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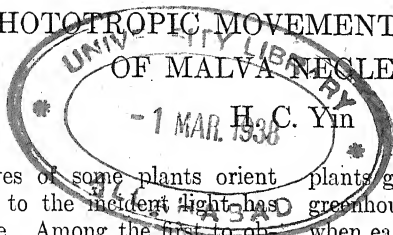
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ERRATA

- Page 83, column 1, lines 22-23.—Substitute "thermal emission" for "reradiation."
- Page 85, column 2, line 41.—Substitute "convection" for "connection."
- Page 90, column 1, line 8 from bottom.—Change to read "16°C. above air temperature."
- Page 91, last line of caption for figure 5.—Substitute "52.5°C." for "19.5°C."
- Page 116, column 1, following third paragraph.—Insert: "***Penstemon amplus*** Aven Nelson, sp. nov.; type number 2075, secured by Aven Nelson and Ruth A. Nelson; roadbank, five miles above Sedona, in Oak Creek Canyon, Arizona, May 23, 1935."
- Page 158, tenth line of caption for figure 2.—Substitute "Aid" for "aid" and "AiD" for "Aid."
- Page 161, column 1, line 18.—Substitute "id" for "iD."
- Page 163, third line of caption for figure 6.—Change to read "Aid × aiD."
- Page 165, column 1, lines 1-3.—Transpose "(G5.3 × C8)2.11" and "(481.13 × C4)10."
- Page 530, column 1, next to last line.—Insert "(Skoog, Broyer, and Grossenbacher, 1938)" between "growth controlling factors" and "stimuli."
- Page 533, column 1, line 39.—Reference should read "Freeland, 1936."
- Page 535, column 1, line 1.—Reference should read "and T. C. Broyer, 1936b."
- Page 535, column 2, line 13.—Delete letter "S" to read "Skoog, F."

DIAPHOTOTROPIC MOVEMENT OF THE LEAVES
OF *MALVA NEGLECTA*¹



25792

THE FACT that the leaves of some plants orient themselves perpendicularly to the incident light has been known for a long time. Among the first to observe the phenomenon were Bonnet (1754) and Dutrochet (1833). Later it was studied by Frank (1870) who gave it the name "transverse heliotropism." Frank thought that light was the causative agent for the movement, but de Vries (1874) believed that several factors working together were responsible—e.g., geotropism, epinasty and hyponasty, and weight of the leaves. The question was settled in favor of Frank's hypothesis by the work of Darwin (1881) and Vöchting (1888). Darwin also proposed the name "diaphototropism" which has since been in general use.

In the beginning of the present century attention was directed to the question of the perception of light by leaves. Haberlandt (1905) founded the lens-action hypothesis which was later disproved by the work of Knipf.

Diaphototropic movements can be observed in the leaves of many plants, chiefly Leguminosae and in flower heads such as the sunflower. They are often mentioned in connection with ecological studies. The physiological side of the question has not been studied except by Vöchting (1888) and Denecke (1924). Vöchting made many interesting observations, the results of which will be discussed later. Denecke was chiefly concerned with the perception of light. His results were of little value as he failed to distinguish clearly diaphototropic movement from other movements of the leaves. As far as is known to the writer diaphototropic movement has not yet been carefully analyzed, and its mechanism remains obscure.

In the present study the leaves of *Malva neglecta* were used. Beside exhibiting the daily diaphototropic movement, these leaves also showed a number of other movements such as epinasty, geotropism and phototropism of the petiole; and sometimes sleeping movements. To avoid confusion it seems necessary at the outset to define the diaphototropic movement: it is the orientation of leaves in such a way that the upper faces of the laminae place themselves perpendicular to the incident light. In fact, the leaves are so sensitive that they, as described by Bonnet, "suivent en quelque sorte le cours du soleil: en sorte que le matin ces feuilles regardent le levant; vers le milieu du jour, le midi; le soir, le couchant" (see Vöchting, 1888).

The present paper attempts: (1) to determine the directive action of light, (2) to differentiate the organs of perception and reaction, and (3) to discover the mechanism of the movement.

EFFECT OF LIGHT ON THE MOVEMENT.—*Observations under normal light conditions.*—Young *Malva*

plants grown in the field were transplanted into the greenhouse. Two to four weeks after transplantation, when each plant had three or four leaves, experiments were started. The daily movement of the leaves were photographed with a Zeiss cinematographic camera which automatically made an exposure every six minutes. The film was then developed, projected, and the measurements were made.

Figure 1 shows the results of such an experiment. The abscissa represents the time in hours starting from midnight. The ordinates give the angles in degrees the leaf planes made with the horizontal, the angle being zero when the leaf faces upward, negative when it faces east and positive when it faces west. The three curves give the movements of three leaves during the whole day. It is clear from the curves that during the night all the leaves faced east and remained in that position until dawn. As the sun rose they turned upward gradually reaching a horizontal position at noon. They then turned further toward the west with the sun, and after the sun had set gradually turned back so that they faced east again before midnight. The straight line represents the change in the hour angle of the sun calculated on the basis that the sun moves 15° an hour from 6 a.m. to 6 p.m. It is seen that the leaves moved with the sun, their leaf blades being always perpendicular to the sun's rays. The oldest leaf (lowest curve) was slightly less sensitive.

The fact that the leaves returned to their morning position (facing east) in the night is particularly interesting. This phenomenon has never been reported before, although it appears quite important in understanding the mechanism of the whole movement. It can not be considered as a sleeping movement since it bears no relation to the insertion of the leaves. Thus leaves joined to the stem on the east side of the plant bend abaxially and leaves on the west side of the plant bend adaxially, while leaves on the north and south sides turn sideways. The movement during the night is also not dependent on the geographic direction. Experiments have been made by turning the plants through 180° just before sundown so that the leaves were now facing east instead of west. After several hours it was observed that the leaves, instead of staying where they were, had turned toward the west—i.e., their original morning position with respect to the plant. In other experiments the plants were turned 90°, and the same result was obtained. This behavior lasted for several days before the plant was adapted to its new position. Evidently the returning movement is an after-effect, an equalization of some kind of strain set up in the day's movement. The morning position appears to be an equilibrium position. One condition, however, has

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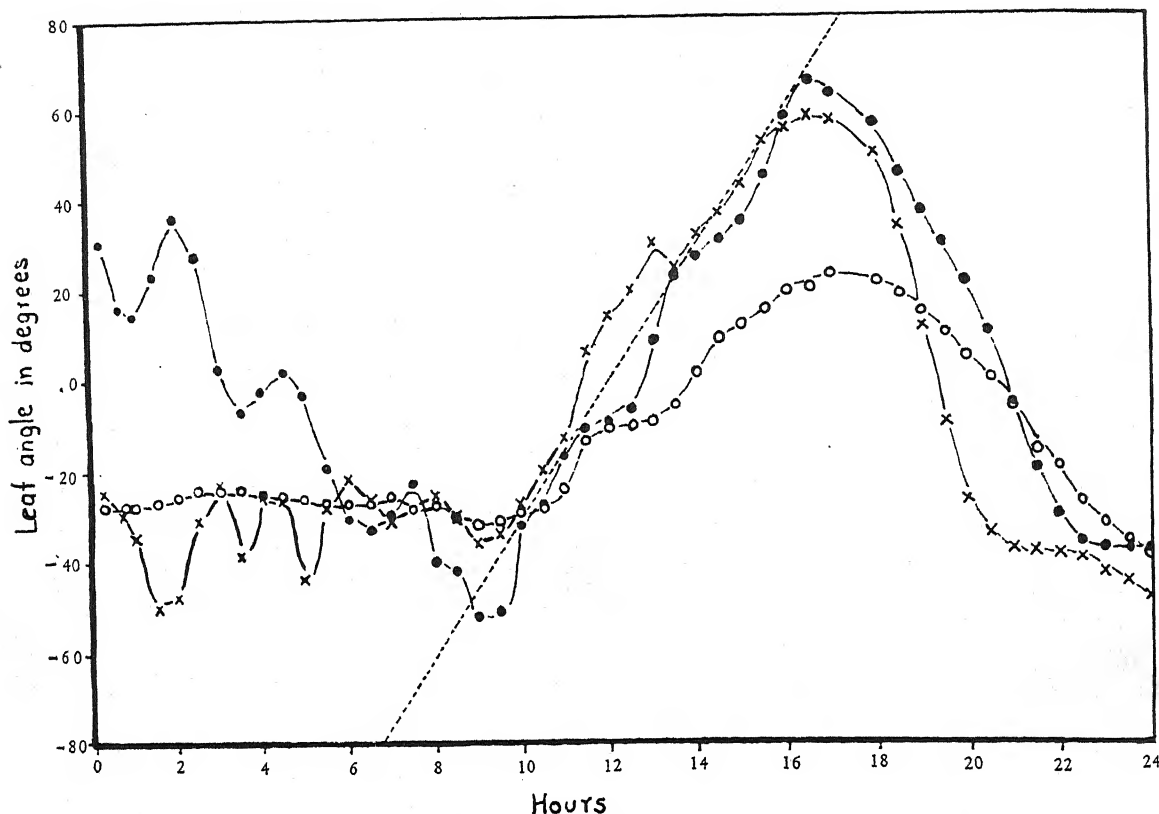


Fig. 1. Diaphototropic movement of *Malva* leaves. o, old leaf; ●, growing leaf; X, young leaf; —, hour angle of the sun.

been found necessary for this returning movement: the plants must have morning light—i.e., they must be placed in such a position as to receive direct light from the sun in the early morning. If they do not receive morning light—e.g., if the plants are grown against an eastern wall where they cannot get direct sunlight until noon—then in the night their leaves will not turn toward the east but will drop down abaxially as in ordinary epinastic movement.

Observations in diffused light.—To find out whether sunlight has a directive influence on the movement a similar observation was made on a rainy day when the sky was overcast and there was no apparent movement of the sun. The results are given in figure 2. Throughout the whole day the leaves remained in a more or less horizontal position. In the morning and in the night they turned slightly east.

Experiments were also carried out with plants enclosed in a tall cardboard screen, which allowed light to fall only from above. An electric light was sometimes used to supplement the daylight intensity. Here again the leaves assumed a horizontal position. It is evident therefore that the movement is not autonomous and is dependent on the direction of the incident light.

Observations in darkness.—Several experiments were done in an underground darkroom where the temperature and humidity were regulated at 24°C. and 85 per cent respectively. At intervals the movements

were measured under orange light by means of a protractor.

The results were not uniform, as the plants showed nutations and nastic movements. A few leaves, however, did show more or less periodic movements, but they were not diaphototropic orientations in the sense that they were not regularly in the east-west direction and that the curvatures were not limited to the upper end of the petioles. They were, probably, comparable to sleeping movements.

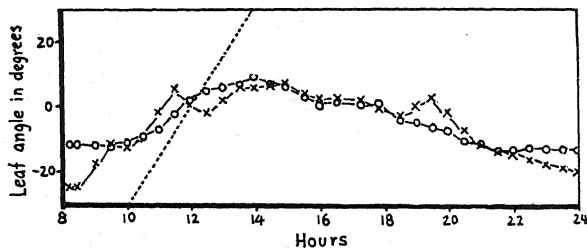


Fig. 2. Movement of leaves in diffused light.

On account of the irregular and compound nature of these dark movements, they can not be easily analyzed. The same thing was observed by Denecke in his work. This seems to be a general characteristic in plants having movable joints.

Observations under colored lights.—In several experiments carried out in the greenhouse, plants were

placed under two Senebier jars filled respectively with solutions of CuSO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$. For three consecutive days the plants were exchanged daily between the bell jars, and the movements of the leaves were carefully followed. The results of one such experiment are shown in table 1.

It is seen that all the leaves showed movements in the white light and under the blue filter, but none under the red filter. The intensities under the two bell jars might have been different, but since this does not constitute an important factor in the movement (Vöchting, 1888), it is justifiable to conclude that the red light is inactive diaphototropically.

TABLE 1. *Diaphototropic movement of leaves of Malva under colored filters.*

Date	April 30	May 1	May 2
Color	Blue	Red	Blue
Plant 1:			
leaf 3	+	—	+
leaf 4	+	?	+
leaf 5	+	—	+
leaf 6	+	—	+
Color	White	Blue	Red
Plant 2:			
leaf 1	+	+	?
leaf 2	+	+	—
leaf 3	+	+	—

ANALYSIS OF THE MOVEMENT.—*The organ of perception.*—To find out whether the lamina or the petiole is responsible for the perception of light, experiments were made as follows. Active leaves were chosen on several plants and their laminae or petioles (including the laminar joints) were shaded from light by means of black paper, rubber tubing, or India ink. Black paper was found to be the most satisfactory. The movements of these leaves were then observed for three consecutive days. The results as summarized in table 2 indicate that the laminae are the perceptive organs. Shading of the petioles does not stop the movement.

TABLE 2. *Diaphototropic movements of leaves with petioles or laminae shaded.*

	Total no. of leaves	No. of leaves showing diaphoto. movement	No. of leaves not showing diaphoto. movement
Controls	12	11	1
Petioles shaded	11	10	1
Lamina shaded	9	0	9
Both petiole and lamina shaded ...	2	0	2

In some plants it is known that the center of the leaf blade—i.e., the part just above the insertion of the petiole—is especially sensitive to light. This, however, is not true for *Malva*. In three experiments fifteen leaves were partially shaded either in the center or in the margin by means of black paper and India ink. All of them showed movement. Therefore the whole blade is equally sensitive to light; as long as a

part is exposed to light, movement can be observed. The same results had been obtained by Denecke (1924).

The organ of reaction.—During the daily movement it was observed that nearly the entire length of the petiole remained stationary. The movement was solely brought about by a sharp curvature of the upper end of the petiole about 3–5 mm. from the leaf insertion.

The active part of the petiole has been called the laminar joint (Pfeffer, 1903). It is thicker than the rest of the petiole and has a peculiar anatomical structure in that it has only one central vascular bundle, while the petiole has six or seven strands arranged on the periphery. The change in the structure is gradual. At 5 to 6 mm. below the lamina the petiolar bundles begin to fuse into a hollow ring. The central pith is then gradually eliminated until at about 2 mm. from the lamina it disappears, resulting in a single solid bundle. On entering the lamina the bundle first becomes horseshoe-shaped and separates into seven strands which emerge into the main veins of the lamina. The joint with a central bundle surrounded by parenchyma is, teleologically speaking, very well adapted to the movement. Serial sections were made from this part of the petiole and are shown in figure 3. The structure of the variation joint in this plant has been observed by Vöchting (1888) and in *Phaseolus* by Pfeffer (1903).

THE MECHANISM OF THE MOVEMENT.—*Relation to growth.*—The next phase of the problem was to find out whether the curvature movement is a result of growth or a variation in turgor. Experimental evidence indicates that this is not a growth movement, because: (1) There is no relation between the grand period of growth and the activity of the diaphototropic movement. Very young leaves do not show this movement despite the rapid growth of their petioles. The nearly mature leaves show the most active movement. Mature leaves, which have practically ceased to grow, still show distinct movement for sev-

eral days before they are shed. (2) There is also no relation between the growing zone and the zone of movement. We have seen that the curvature takes place only in the laminar joint, but the zone of rapid growth is always below the joint. Measurements showed that in young leaves the growing zone is in the lower part of the petiole; it moves upward as the leaves grow older but never reaches the joint.

Extension and contraction of the moving laminar joint.—To verify the conclusion that the movement is not due to growth, the variations in the lengths of the two sides and of the median line of the joint were measured during the movement. The measurements were made by means of a micrometer. The joint was about 4 mm. long, the accuracy of the measurements being within 0.5 micrometer divisions (1/30 mm.).

The results are shown in figure 4. While the length of the east side of the joint increased steadily from morning until 4 p. m., the west side contracted proportionally, and consequently the joint curved toward the west. After sunset the phenomenon was reversed, both sides quickly regained their original lengths, and

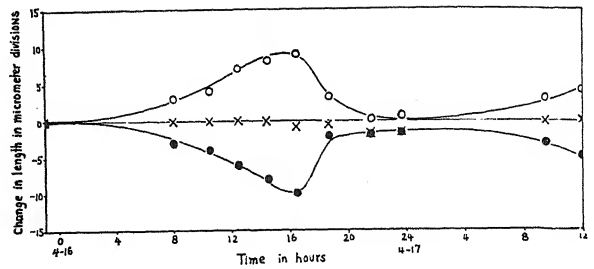


Fig. 4. Extension and contraction of two sides of laminar joint during diaphototropic movement. O, east side; ●, west side; X, middle line of laminar joint.

Leaf insertion

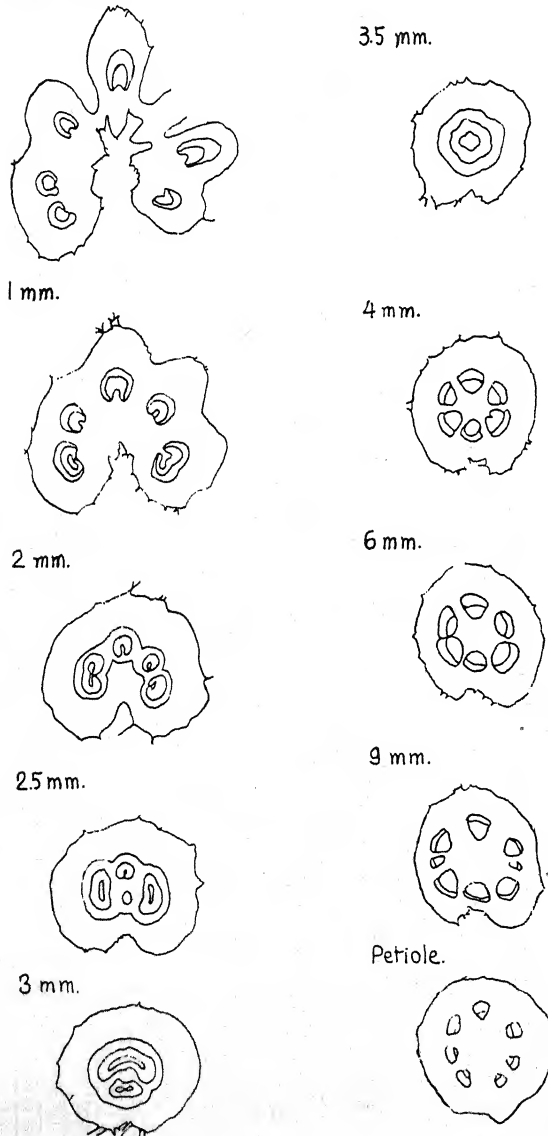


Fig. 3. Vascular structure of laminar joint and petiole at different distances (approx.) from insertion of lamina.

the leaf faced east again. During the whole movement the length of the middle line of the joint remained unchanged. It is evident, therefore, that the curvature is not due to a differential growth, but to the reversible expansion and contraction of the two sides of the joint (Pfeffer, 1903).

OSMOTIC RELATIONS OF THE JOINT.—To get a complete picture of the turgor relations between the two sides of the joint, it would be necessary to determine both the suction force and the osmotic pressure of the cells (Weidlich, 1930). Both determinations, however, were found to be unsatisfactory and not accurate enough for this purpose. Therefore only the osmotic pressure at incipient plasmolysis (O_p) was measured. The plasmometric method of Höfler (see L. Brauner, 1932) was used instead of the ordinary method of determining O_p because the cells were colorless, and thus the plasmolytic condition could only be ascertained in its advanced stage (Ernest, 1935). Each experiment consisted of several determinations of O_p at different times of the day. The same plant was used in each experiment and one joint was used for each determination. The joint was sectioned and placed in sucrose solutions previously found to be isotonic or slightly hypertonic by the usual method of interpolation. A group of ten plasmolyzed cells in the cortex was drawn with the aid of a camera lucida. The area of the protoplast (A_p) and that enclosed by the cell wall (A_c) were measured with a planimeter. The value of O_p was then calculated by means of the following expression, where V_p represents the volume of the protoplast and V_c the volume of the cell at incipient plasmolysis.

$$O_p = M \frac{V_p}{V_c} = M \left(\frac{A_p}{A_c} \right)^{3/2}$$

These values of O_p were transformed into atmospheres according to the table given by Molz (1926). Several experiments were made. The results of two such experiments are given in table 3.

Since we are here dealing only with the undifferentiated, thin-walled cortical cells in the close vicinity of vascular tissue, it seems reasonable to consider that the suction force is the same in the two sides and that O_p is the primary factor determining the turgor pressure and the volume of the cell. If this is true, the mechanism of the movement becomes explicable. It

TABLE 3. O_x of cells of two sides of laminar joint.

Time	East side					West side					Diff. O _g
	Sucrose M	A _p /A _c	V _p /V _c	MV _p /V _c	O _g atm.	Sucrose M	A _p /A _c	V _p /V _c	MV _p /V _c	O _g atm.	
Exp't. 1. Greenhouse plant											
9:45 A. ...	0.90	0.872	0.814	0.733	22.8	0.85	0.880	0.826	0.702	21.6	1.2
12:00 N. ...	0.90	0.860	0.798	0.718	22.2	0.85	0.845	0.777	0.661	20.0	2.2
3:00 P. ...	0.95	0.921	0.884	0.840	27.2	0.90	0.893	0.844	0.760	23.8	3.4
6:00 P. ...	0.95	0.862	0.801	0.761	23.8	0.95	0.900	0.854	0.811	26.0	-2.2
9:00 P. ...	0.85	0.898	0.851	0.724	22.5	0.85	0.880	0.826	0.702	21.6	0.9
11:15 P. ...	0.90	0.873	0.816	0.734	22.9	0.90	0.871	0.813	0.732	22.8	0.1
Exp't. 2. Field plant											
9:30 A. ...	0.95 ^a	0.789	0.701	0.666	20.2	0.85	0.842	0.773	0.657	19.9	0.3
1:30 P. ...	0.80	0.840	0.770	0.616	18.3	0.80	0.740	0.637	0.509	14.6	3.7
6:00 P. ...	0.80	0.845	0.777	0.622	18.6	0.80	0.893	0.844	0.676	20.6	-2.0
10:30 P. ...	0.85	0.860	0.798	0.678	20.6	0.85	0.847	0.780	0.663	20.1	0.5

^a Concentration too high; cells reached "ball" stage of plasmolysis.

can be seen from table 3 that during the day the O_x of the cells in the east side of the joint was always higher than that in the west side; consequently these cells were larger and the joint curved toward the west. At sunset the difference in O_x was reversed and so was the returning movement. Finally at night the O_x 's were equal and the movement stopped.

Let us now compare the values of O_x at different times of the day. At midnight the O_x of the two sides was the same. In the morning, while the O_x in the east side remained constant, it decreased in the west side. In the afternoon the O_x increased in both sides, the increase being more at first in the east side and then in the west side. In the night the O_x on both sides dropped off again.

Assuming the volumes of the cells at incipient plasmolysis to be constant, these changes of O_x indicate the changes in the amount of osmotically active substances of the cell, brought about either by a change in the permeability of the membrane or by a reversible hydrolysis or precipitation of substances in the cell. The latter is more probably the case. The stretchability of cell wall is not considered here because in no case is it known that stretchability can change reversibly; nor is a decrease of wall stretchability known to produce contraction of the cell.

CONCLUSIONS AND DISCUSSION.—In the first part of the investigation it was shown that the direction of light is the causative factor for the diaphototropic movement of the leaves of *Malva*. These results confirm the hypothesis proposed by Frank (1870) and experimentally established by Darwin (1881) and Vöchting (1888). By the use of a clinostat these authors proved that geotropism, epinasty, etc., have nothing to do with the movement, and light alone is important.

For the perception of light the leaf blade is responsible. In this respect *Malva* is comparable with such plants as *Tropaeolum* (Denecke, 1924) and unlike *Fuchsia* and *Phaseolus* (Pfeffer, 1903). The present study shows only that the movement is caused by the direction of light. Whether the plant perceives

the direction of light as such (Haberlandt 1905) or whether the movement is caused by the small differences of intensity of light on the blade is not known. That the first hypothesis is probably wrong has been indicated by the work of Kniep. The second view, though tenable in the case of concave leaves, is inadequate for the flat leaves of *Malva* where the intensity difference is extremely small. Definite conclusion, however, must await further experimentation.

The mechanism of the reaction is of particular interest to us. Our results confirmed Vöchting's finding that the laminar joint is alone concerned in the movement. The bending of the joint during movement is not due to growth because, while one side extends, the other contracts. It is due to a reversible change in turgor on the two sides as shown by osmotic pressure measurements. The change in osmotic pressure may be due to either a change in permeability of the protoplasm (Lepeschkin, 1934) or the formation and precipitation of the osmotically active substances (e.g., starch \rightleftharpoons sugar). The permeability change is probably the cause of the quick movements, e.g., in *Mimosa* (Pfeffer, 1903) and in *Sparmannia* (Bünning, 1930); the anatonosis and precipitation of the osmotic substances are probably mainly responsible for the slow movement, such as we have here in *Malva*.

Recent work on the mechanism of turgor movements has been chiefly done on *Phaseolus*. The primary leaves of this plant exhibit both photonastic and sleeping movements. Unlike *Malva* the laminar joints of *Phaseolus* are found to be dorsoventrally dissimilar (Weidlich, 1930; M. Brauner, 1933). The osmotic changes during the nyctinastic movement have been studied by Weidlich (1930), who found that the movement is due to the alternate increases and decreases of osmotically active substances in the upper and lower sides of the joint. These results are therefore comparable to those we have found in *Malva*. Later work by Brauner (1932), Bünning (1933), and Lepeschkin (1934), however, inclined to explain such movement on the basis of changes in the permeability. Since no conclusive data have ever

been presented, the permeability hypothesis can not be regarded as established.

As to how the stimulus is conducted from the lamina that perceives the light to the joint that gives the reaction, nothing is as yet known. That the conduction is through the veins has been shown by Denecke (1924). In our experiments with artificial light we found that the reaction time was long (more than half an hour). This suggests that the rate of conduction is rather low. In this respect it differs from the seismonastic turgor movements of *Mimosa* or *Sparmannia*, the reaction time of which is a matter of a few seconds. Perhaps the conduction here is of a chemical nature comparable to the slow conduction of *Mimosa* (Fitting, 1930) or in grass seedlings (Went, 1928).

SUMMARY

The daily diaphototropic orientation of the leaves of *Malva neglecta* was studied with the aid of a motion picture camera. The leaves were found to follow closely the course of the sun, with their faces always perpendicular to the incident rays. After sunset they turned back to their original morning positions (facing east). No such orientations of leaves have been found in evenly diffused light and in continuous darkness. The direction of the incident light is therefore important.

Plants kept in daylight under a red filter showed no diaphototropic movement, but they showed such a movement with the shorter wave lengths of daylight.

Leaf-laminae were found to be the organs of perception for light.

Diaphototropic movement (curvature) was found to be limited to the upper 4 or 5 mm. of the petiole. This part (laminar joint) has a different anatomical structure from the rest of the petiole. It has a central bundle instead of several peripheral ones.

The curvature is not due to growth because: (a) no relation exists between the grand period of growth and movement activity; (b) no relation exists between the growing zones and the movable part (laminar joint). The curvature was found to be the result of extension and contraction of the two sides of the joint. These changes in length are completely reversible.

By measuring the osmotic pressure (O_x) of the cells at different times of the day, it can be shown that the movement is due to a difference in turgor between the two sides of the joint.

The results are discussed briefly in connection with the literature on turgor movements.

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REGENERATION IN *CRASSULA MULTICAVA*¹

Ilda McVeigh

CRASSULA MULTICAVA Lem. (*C. quadrifida* Bak.) is a native of South Africa. According to Figdor (1918) this plant may be propagated not only by buds which are formed regularly in the axils of the bracts of the inflorescences but also by buds induced to form on leaves. The latter are formed after severance of the main vein both on leaves still attached to the parent and on those detached and placed on moist sand. Figdor reported that the new structures formed on the leaves arise from callus, although he failed to give the histological details. The purpose of this investigation is to make a histological study of the origin of the new plants formed on the leaves.

EXPERIMENTAL PROCEDURE.—Material for this study was taken from cuttings obtained from the Missouri Botanical Garden and from the New York Botanical Garden. Entire leaves and portions of leaves were placed on moist filter paper in Petri dishes. Others were placed with the wounded surface in moist sand. At intervals of one or two days the material was examined with the binocular microscope, and basal portions of leaves or parts of leaves were removed and placed in chromo-acetic solution. The paraffin technique was used; the material was sectioned at 8 microns and stained with Heidenhain's iron-alum haematoxylin. Both transverse vertical and longitudinal vertical sections were made.

GROSS MORPHOLOGY.—*Crassula multicava* is a low herb with more or less decumbent base. The stems are succulent and bear opposite, decussate leaves (fig. 1). The petioles are short, sometimes so short that the leaves appear almost sessile, and petioles of a pair are joined. The fleshy, glabrous leaves are obovate with very obtuse tips and a tapering base. Numerous hydathodes give the leaves a more or less spotted appearance.

The maximum size of the leaves is approximately 7.5 by 4.0 by 0.2 cm. Both lamina and petiole are composed largely of parenchymatous tissue. In this tissue there are occasional cells which are filled with tannin and are stained black in the preparations. Characteristic of both petiole and lamina is a layer of subepidermal cells almost all of which are filled with tannin (fig. 5, 8). This layer of cells aids in the study of the slides. The cells of the upper and lower epidermis are alike in cross section. The mesophyll is composed entirely of spongy tissue; there is no palisade layer (fig. 11). In the lamina these cells contain many chloroplasts, especially near the margins of the leaves; in the petiole the chloroplasts are less numerous.

Commonly there are five leaf traces, one large central vein with two smaller lateral veins on each side of it. Occasionally there are seven traces. The phloem is arranged in small groups of cells on the

lower side. In the lamina there is a single large midvein with several smaller branch veins. From these branches a reticulum of minor veins is formed; some of these end in the hydathodes.

DEVELOPMENT OF NEW PLANTS.—The cells of the wounded surfaces of leaves placed in moist chambers soon collapsed, and the cells underneath these divided in a plane parallel to the cut to form a periderm (fig. 9, 10). Ordinarily very little, if any, callus was formed on leaves under such conditions, while those placed in moist sand usually formed a considerable amount.

Entire leaves produced new plants from both the upper and lower sides of the petiole near the cut surface; however, the majority arose from the lower side (fig. 2, 3). Small protuberances, visible under the binocular microscope, appeared near the injured region of the petioles of some leaves six days after their removal from the parent plant. The roots and the shoot with the first pair of leaves soon developed from each mound. A leaf usually produced three or four plants (fig. 3). The basal portions of some leaves were removed as many as four times and each time the leaves showed evidence of regeneration.

Leaves which were divided into halves by a cut perpendicular to the longitudinal axis were capable of regeneration from the basal portion of each. On the basal half, new plants were formed on the petiole as described for entire leaves; no regeneration occurred near the wounded area at the apex. Plants were also formed on the lamina of the apical half, near the cut and usually not far from the midrib (fig. 4). None was observed on the upper side. Leaves cut crosswise into four pieces had not formed plants after 110 days in a moist chamber.

ANATOMICAL FEATURES.—The new plants were found to arise from epidermal cells. Cell divisions were observed within two days after separation from the parent plant (fig. 12). The first divisions were periclinal (fig. 12, 13); later anticlinal divisions also occurred (fig. 14). In some material the original cell wall could be distinguished for a period of time (fig. 14, 15). The divisions extended back a distance from the wounded surface. These cells had large nuclei and much cytoplasm; they stained in a manner characteristic of meristematic tissue. The cells of the mesophyll near the wound lost their chloroplasts but only those in the immediate vicinity of the injured cells underwent division; from these a periderm was formed (fig. 10). After meristematic activity was initiated in the epidermis, divisions occurred rather rapidly, and by the end of four days a layer of tissue 3 or 4 cells thick was formed (fig. 14). Activity was not limited to a group of a few epidermal cells but was continuous in the epidermis around the entire petiole near the wounded surface (fig. 15, 16). Very soon groups of cells projected above the surrounding

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cells because of the more rapid divisions in certain areas (fig. 6); from these groups the new plants were differentiated.

Usually the projecting mound of tissue grew directly into a root (fig. 8). Differentiation of the histogens was detected. Vascular connections with the conducting system of the parent plant were formed by division of the parenchyma cells. In figure 8, although some of the xylem cells had already formed spiral thickenings, the walls of the original parenchyma cells from which they were formed are still clearly distinguishable. Several cells were formed from a single parenchyma cell. Soon two small protrusions, the first pair of leaves, appeared. These are shown in figure 7—a portion of a longitudinal section of a petiole in which the axis of the new plant was sectioned in a longitudinal plane. At one end is the primary root and at the other the two primary leaves. The vascular connections with the parent plant are not shown in this section.

Sometimes instead of the bud developing directly as described, there was first developed a considerable root system. The primary root increased in length, formed root hairs and often branches; the basal portion enlarged, and from this several roots originated. Then on this swollen basal portion next to the parent leaf or directly from the lamina nearby, one or more buds, each with two primary leaves, were formed (fig. 9). Usually only one of these buds continued development. In this type of development very often the tip of the primary root died, and this was followed by the enlargement and the subsequent development of several roots.

New plants arose from the lamina as they did from the petiole but more slowly. The normal structure of the lamina is shown in figure 17. Figure 18 is a transverse vertical section of a lamina near the wounded area eleven days after it was placed in the moist chamber. In the vicinity of the wound the chloroplasts had disappeared from the parenchyma cells. The cells of the lower epidermis underwent both periclinal and anticlinal divisions. The walls of the original cells were still distinguishable. Meristematic activity was greatest in the vicinity of the midrib; it extended in both directions about one half the distance from the midrib to the margin. No divisions were observed in the upper epidermis of the lamina. The differentiation of the plant axis from the meristematic tissue derived from the epidermis was like that in the petiole. Figure 10 is a cross section of the lamina showing a longitudinal section of a young plant. Only one of the primary leaves is visible in this section.

I have not investigated the origin of buds which arise in the axils of the bracts of the inflorescences (Figdor, 1918). The plants available have not yet produced flowers. Figdor reported also the formation of new plants from the wounded area of attached leaves after about six months. I have wounded leaves in various places—along the margins, in the region of the midrib and larger veins, and between the larger

veins. After a period of 7 weeks macroscopic examination revealed no evidence of regeneration.

DISCUSSION.—Histological studies show that vegetative reproduction of members of the Crassulaceae occurs in different ways. Naylor (1932), Yarbrough (1932), and others report the presence of definitely organized, dormant embryos in the notches of leaves of *Bryophyllum calycinum* Salisb. These embryos originate while the entire leaf is still meristematic. They normally resume development only after the leaf has been detached from the parent plant. The origin and development of new plants from the leaves of *Kalanchoe tubiflora* Hamet (Clamp, 1934) and some other species is similar to that described for *B. calycinum*, except that the embryos continue their development and grow out into new plants while the leaf is still attached to the parent plant. According to Stoudt (1934), the plant formed vegetatively on a leaf of *Byrnesia Weinbergii* Rose arises from a dormant meristem located at its base. No differentiation of the meristem into a new plant occurs while the leaf is still attached to the parent plant. Hence, new plants which arise from the leaves of *Bryophyllum*, *Kalanchoe*, and *Byrnesia* originate from residual primary meristems and would therefore not come in the category of regeneration.

Yarbrough (1936) has described the origin of new plants from a secondary meristem arising from vacuolated, differentiated parenchyma of the leaves of *Sedum Stahlia* Solms. From the evidence presented, I am not convinced that the possibility of the new plants arising from residual primary meristem at the base of the petiole has been entirely eliminated. It seems clear, however, that the new plants which originate from leaves of *Crassula multicava* arise from a secondary meristem derived from mature epidermal cells. The possibility of the new plants being derived from a primary meristem present at the base of the petiole has been eliminated by removing the basal portion of the petiole. In the leaves of *C. multicava* there is not a continuity of meristematic tissue, but severance of the leaf brings about changes in the epidermal cells followed by a resumption of mitotic activity and the subsequent differentiation into a new plant. Although a wound callus may be formed, it takes no direct part in the origin and development of the new plant, notwithstanding Figdor's statement to the contrary.

A cytological study of the epidermal cells concerned in the regeneration of *C. multicava* is desirable. What changes occur in the cytoplasm and nuclei of these cells as they become meristematic? The answer to this question might be of value in determining why a cell dedifferentiates.

C. multicava resembles *Saintpaulia ionantha* Wendl. (Naylor and Johnson, 1937) and *Begonia Rex* Putz. (Regal, 1876; Hansen, 1881; Hartsema, 1926) in the origin of buds from epidermal cells. However, the roots of *Saintpaulia* and *Begonia* originate endogenously, while those of *C. multicava* arise exogenously from epidermal cells. So far as is known, this is the

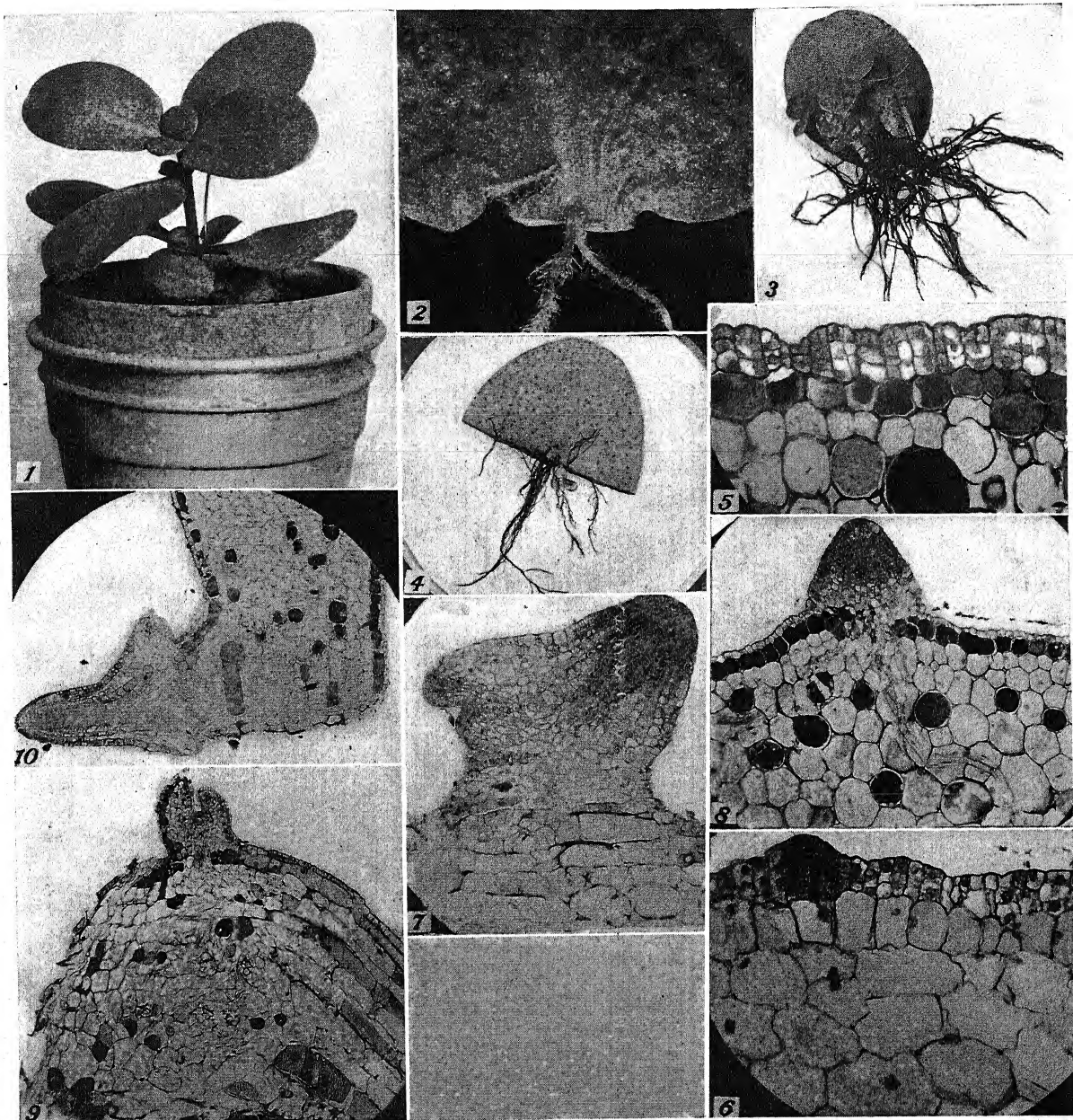


Fig. 1-10.—Fig. 1. Plant of *Crassula multicava*.—Fig. 2. Leaf after 20 days in moist chamber. Young plant with two roots and two leaf primordia has developed from the upper epidermis.—Fig. 3. Leaf showing four plants developed from the lower epidermis of the petiole, after 7 weeks in moist sand.—Fig. 4. Portion of leaf showing regeneration from the lower epidermis of the lamina. Note the formation of plants at some distance from the midvein, after 7 weeks in moist sand.—Fig. 5. Portion of cross section of petiole showing divisions in the epidermis after 4 days. Note the subepidermal cells and the scattered parenchyma cells which are filled with tannin.—Fig. 6. Divisions in the upper epidermis after 7 days. Note the group of cells which protrudes beyond the adjacent cells.—Fig. 7. Portion of longitudinal section of petiole showing a longitudinal section of a young embryo. Note the root primordia and the two leaf primordia. After 11 days.—Fig. 8. Portion of cross section of petiole showing young root primordium. Note the divisions of the parenchyma cells to form vascular connections. The original walls of the parenchyma cells can still be distinguished. After 20 days.—Fig. 9. Portion of cross section of petiole showing shoot primordium with two primary leaves. Note vascular connections. After 21 days.—Fig. 10. Longitudinal vertical section of lamina showing a longitudinal section of an embryo. Only one leaf primordium is visible. Note the periderm formation; also the absence of chloroplasts in the parenchyma cells near the wound. After 36 days.

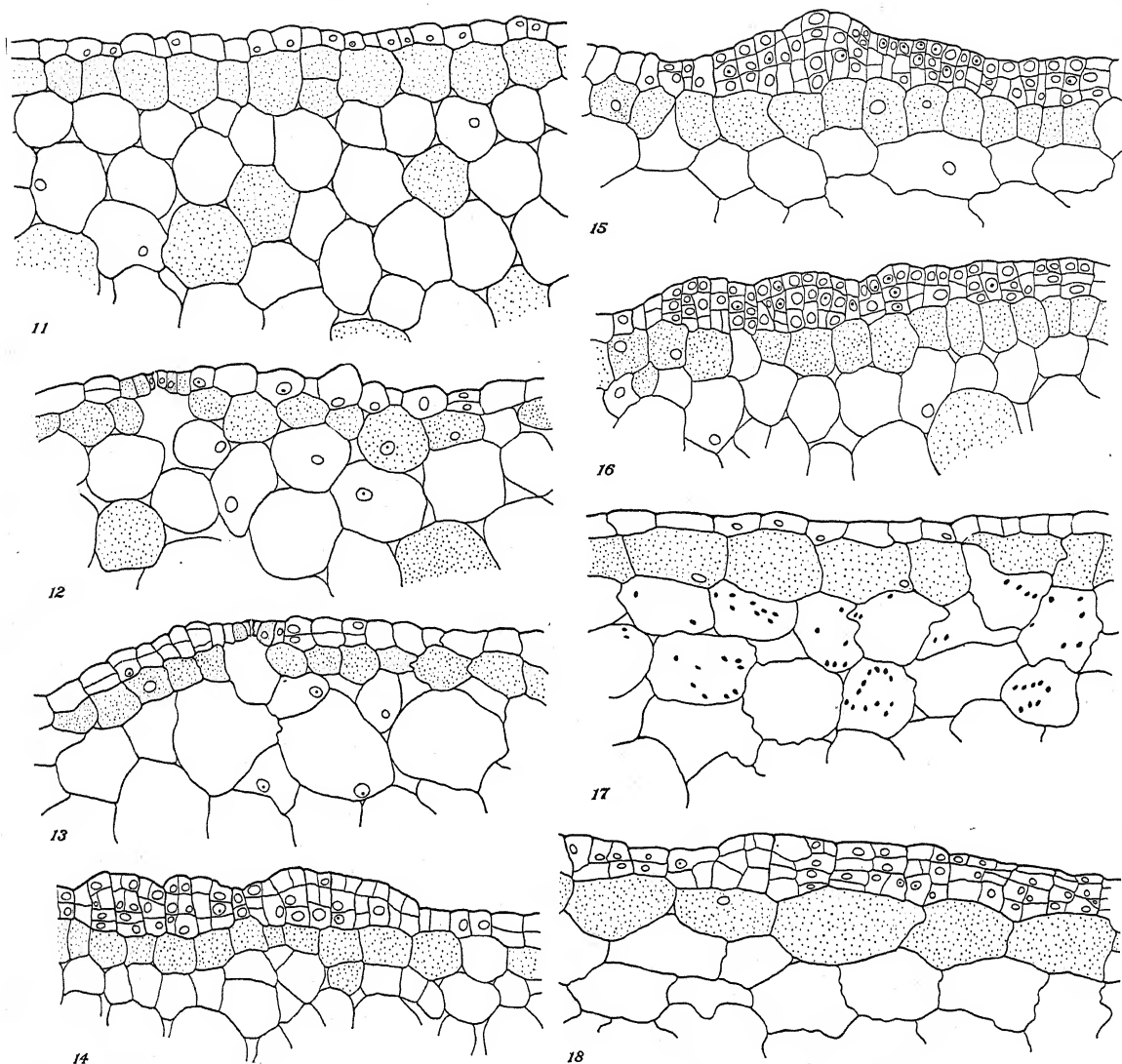


Fig. 11-18.—Fig. 11. Portion of cross section of petiole showing normal structure. Stipples indicate presence of tannin.—Fig. 12. Portion of cross section of petiole showing first divisions in lower epidermal cells. After 2 days in moist chamber.—Fig. 13. Same as fig. 12 after 3 days in moist chamber. Original cell walls are distinguishable.—Fig. 14. Further divisions after 4 days. Some of the original walls can still be distinguished. Groups of cells protrude beyond adjacent cells; from such groups new plants arise.—Fig. 15, 16. Portions of cross section of petiole showing normal structure. The lower epidermis is shown.—Fig. 17. Portion of cross section of lamina epidermis of lamina after 11 days.

first report of a species in which the entire new individual originates from mature epidermal cells. Divisions of the underlying parenchyma cells result in the formation of vascular connections with the parent leaf. Epidermal cells take part in the formation of new plants from the hypocotyl of *Linum* (Crooks, 1933) and from the leaves of *Drosera* (Vickery, 1933). Other examples are recorded in the literature.

Specialized cells other than epidermal cells may give rise to new plants or new plant parts. From accounts given, the following types of cells may be concerned:

parenchyma, endodermis, pericycle, vascular ray cells, and immature xylem cells. Very little attention has been given to the cytological changes which occur in these cells.

SUMMARY

New plants are produced from the entire detached leaves or portions of leaves of *Crassula multicava* after about three weeks in moist chambers.

Both roots and buds originate from epidermal cells not far from the wounded surface. So far as is known, this is the first report of a species in which an entire new individual arises from mature epidermal cells.

Vascular connections with the parent leaf are established by divisions of the parenchyma cells between the new plant and a vein of the leaf.

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A COMPARISON OF THE FILAMENTOUS IRON ORGANISMS, CLONOTHRIX FUSCA ROZE AND CRENOTHRIX POLYSPORA COHN¹

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IN MY study of an iron organism which I recently obtained from "hard" water supplied to a section of Brooklyn by artesian wells, I have encountered the problem of accepting or rejecting *Clonothrix fusca* Schorler as a legitimate species. This organism, described by Schorler in 1904 without illustrations, has been rejected by Cholodny (1926), who considers it a *Crenothrix*. Another organism, bearing the same name, but described as a blue-green alga by Roze in 1896, is recognized by Nauman (1921). Schorler does not mention Roze's organism, and the literature of today also overlooks it. Cholodny suggests that the form called *Clonothrix fusca* Roze by Nauman is the same as his *Leptothrix crassa* described in 1924. The evidence does not seem to me conclusive on this point, since Cholodny's description (1924) is not wholly clear. The branching which he describes as "offenbar falsch . . . d.h. dadurch zustande kommen dass Schwärmer oder Sporen sich an die Oberfläche der alten Fäden fest sitzen und hier auskeimen," is obviously not the "false branching" described by Cohn (1875) for *Cladothrix dichotoma*, whose description is still the accepted one for this term. In *Crenothrix*, however, which he considered an unbranched form, Cohn (1870) described the condition above referred to by Cholodny. Since Schorler and Roze referred to the false branching as like that of Cohn's in *Cladothrix dichotoma*, in describing their organisms, it is manifestly an error on the part

of Cholodny to refer their organisms to Cohn's unbranched genus *Crenothrix*.

I have found both *Crenothrix polyspora* Cohn and this falsely branched organism, which I am calling *Clonothrix fusca* Roze, in the water supplied from the house tap. In the summers of 1934, 1935, and 1936, *Clonothrix* appeared in sufficiently large dark brown clumps for the filaments to be easily visible to the eye. In December, 1936, both *Crenothrix* and *Clonothrix* appeared in great quantity during a period of unseasonably warm weather, and spore production was then observed. The organisms described by Roze and Schorler and also one reported as *Clonothrix fusca* Schorler by Ruttner (1906) were found in similar situations. In the United States, Harder (1919) mentions the occurrence of *C. fusca* Schorler on the walls of certain underground aqueducts that conduct water from lakes to city reservoirs, but he does not specify the place. He found *Crenothrix* in the hard water of the city of Madison, Wisconsin. *Crenothrix* has been mentioned as growing with *Clonothrix* by Molisch (1910) and Lieske (1922), and Lieske's figure 32, page 49, shows both *Crenothrix* and *Clonothrix*, although he calls it developmental stages of *Clonothrix*. *Crenothrix polyspora* has been reported many times in the literature and has been well figured by Cohn (1870), Zopf (1879), Smith (1903), Rullman (1907, 1912), and Harder (1919), but there are only meager representations of *Clonothrix fusca* (Molisch, 1910; Lieske, 1922; Nauman, 1921), and these are unsatisfactory in one or more respects. Roze shows the vegetative filaments of his organism well, but he has

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apparently mistaken some other organism as the reproductive condition.

I have not yet been able to grow these organisms in culture, although I have tried 2 per cent manganese peptone solution and agar. Abundant material from the original source has been available for study. Slides (whole mounts) have been made by mounting the unstained or stained filaments in glycerine and sealing in balsam by Diehl's double cover-slip method (1929). The sheaths of the unstained filaments of *Clonothrix*, when mounts are made in glycerine, retain their brown color for four months, but after that become colorless. Mounting them in lactophenol caused them to lose their color immediately. When stained with aceto-carmin, gentian violet, or polychrome methylene blue, the brown color likewise disappears from the sheath, which is left faintly but clearly outlined, while the cells become purplish red, violet, or blue, respectively, according to the stains used.

My study has revealed certain important points of contrast between these two organisms. The *Clonothrix* filaments were not found growing attached, but they always show a distinct but very gradual loss in color intensity and width as they taper to the more or less colorless apical portion. The older portions are dark brown. Long branches are common, but short branches in a whorl of three are sometimes observed, as described by Schorler and figured by Molisch. The branches may be alternate or opposite each other (fig. 9a) with a tendency toward profuseness near the tip of the filament, especially when spore formation is in progress (fig. 6). The sheaths of branch and main filament are closely and firmly attached, although in the apical portions, due to the thinness and delicacy of the sheaths, they appear easily detachable.

It is only in the younger portions of the filaments that the origin of the branches may be observed. Figure 3 shows a cell *a* tangentially applied to the cell below it, suggesting that *a* was the original apex cell and has been displaced laterally by the cell behind it pushing through the sheath, which is very flexible in the younger portions of the filament. The sheath is not always visible around the end cells of a filament, especially in living, unstained material, but its presence was demonstrated uniquely in the specimen drawn. A camera lucida sketch was made as usual and then left for an hour before inking. During the interruption a change appeared in the position of the cells, so that on again observing the specimen, I noted spaces between cells which had previously been in contact with each other, and at these places, marked *x* on the drawing, the sheath became distinctly visible, as indicated by the sketches above each point *x* on the filament.

This is the type of branching described by Cohn for *Cladothrix dichotoma*, and it also coincides with the descriptions of the origin of false branches in the Scytonemataceae and Rivulariaceae of the blue-green algae (West and Fritsch, 1927; Tilden, 1935). Roze

clearly described and figured branching similar to mine in his *Clonothrix fusca*, and West and West (1898), in describing a narrower form than Roze's, which they called *Clonothrix gracillima*, stated, "The false ramification of this plant is most peculiar; the apparent branches seem to be merely tangentially applied at their bases to the primary filaments, and are of the same thickness. The branches appear to arise by the lateral protrusion of a few cells from the sheath, these cells remain attached laterally and grow quite independently into long flexible filaments." The fact that later West (1904) decided that *Clonothrix gracillima* was one of the Schizomycetes of the genus *Cladothrix* is further evidence that it should not be considered *Crenothrix*. Nauman's and also Lieske's figure of *Clonothrix fusca*, which the former designates *C. fusca* Roze and the latter *C. fusca* Schorler, are both branched forms, but it is impossible to decide from their figures whether the branching is true or false. Lieske's figure, however, shows the numerous short branches near the tips of the filaments, like those I have observed (fig. 2, 3), although his cylindrical cells have not yet begun to divide up into spores.

Crenothrix polyspora Cohn is an unbranched filament (fig. 8), but it sometimes presents an appearance (suggested by Cholodny (1924) in his *Leptothrix crassa*) which should not be confused with the false branching described above. This is the development of tufts of young filaments along the sides of older filaments, which Cohn described as the germination of free gonidia or spores adhering to the surface of older filaments, and which has been figured repeatedly since then (Zopf, Rullman, Harder). These later investigators interpreted the germination as taking place from gonidia inside the sheath. In my material I find these colorless tufts of young *Crenothrix* filaments adhering to the surface of the older portions of the *Clonothrix* filaments, which were unmistakable on account of the difference in shape of the cells of these two forms and on account of the brown sheaths of the *Clonothrix* filaments. The short plants could not have been produced by germination of spores inside the sheath, since, in the process of staining, the sheath becomes transparent, and the vegetative cells become clearly visible. These vegetative cells are long and cylindrical, in contrast to the shorter more quadrate cells of *Crenothrix* (compare fig. 8 with fig. 9). The *Crenothrix* filaments taper in a characteristic way toward the base or point of attachment, and my figure also shows this. They were colorless in my material, with the transparent sheaths clearly visible and of the same thickness from base to apex. They are also described by Harder (1919) as generally colorless, although he states that older filaments have a thick coating of ferrie hydroxide. From his figure 2, this appears to be around the sheaths, encasing them in a mass, and is in marked contrast to the colorless sheaths in his other illustrations.

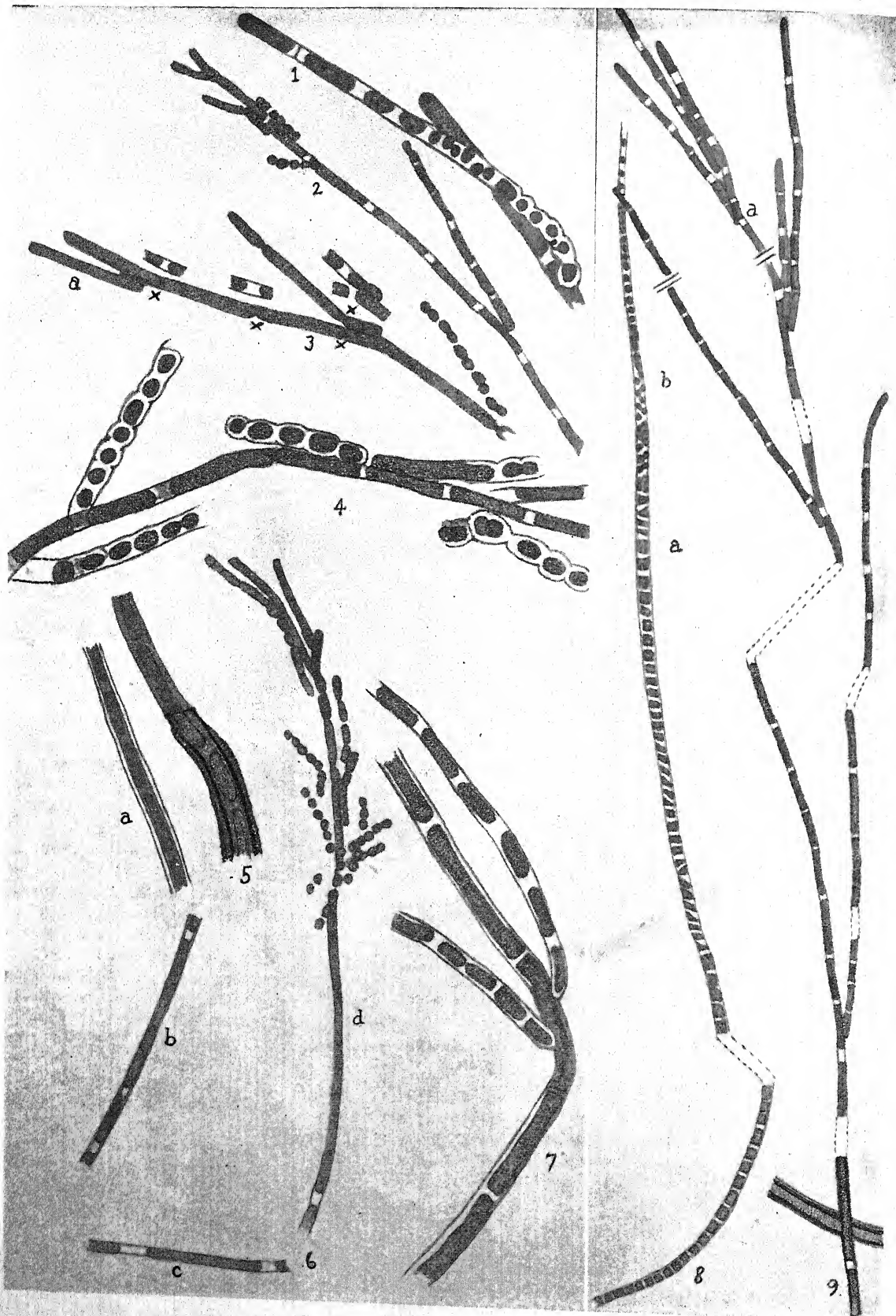
The sheath of the *Clonothrix* filament appears to be of a mucilaginous consistency, at least in the younger portions, where it is colorless, transparent,

and so thin as to be practically invisible. It is extremely difficult to dislodge these filaments from a platinum needle or loop; I have had better success in using a pipette in transferring material. Progressing backward from the colorless portion of these long and flexible filaments, the sheath increases in thickness and becomes yellowish in color, until finally in the older portions the yellow deepens to a rusty brown and then to a blackish brown. The light yellow portion is sufficiently transparent to reveal the cells, but in the older parts, where apparently more hydroxide has been deposited, the cells can be seen with difficulty. These older portions are thicker and more brittle and frequently show cracks in the sheath. Figure 6 comprises sections selected from a filament too long to represent in its entirety. Section *a* represents the older portion where the sheath is thickest, section *b* the next oldest, and *c* and *d* the younger portions where the sheath is barely visible. The thinness of the sheath in these portions contrasts sharply with the portion *a*, where a double line in the drawing represents the sheath, about $0.8\ \mu$ in thickness. This material was stained in aceto-carmin, which removed all color from the sheath immediately. In living material, when the width of a filament was measured, I found the older darker portions to measure $5.6\ \mu$ and $6.4\ \mu$, the lighter portions $4.8\ \mu$, and the portion first beginning to show the yellowish color, $3.2\ \mu$. It is the sheath of the filament which increases in thickness toward the older portions, and there is very little difference in the width of the cells, as may be seen in figure 6. Such a portion, as figure 6*a*, measured $4\ \mu$ across the filament, the cells measuring $2.4\ \mu$, and the sheath extending $0.8\ \mu$ on either side. At the tip of this filament (fig. 6*d*), where it is impossible to see the sheath except between the cells when they have separated, the filament measures about $2\ \mu$, the same as the width of the cells. Compared to the width of the living filaments in the wider portions ($5.6\ \mu$ – $6.4\ \mu$), the stained filaments show a loss in thickness when the brown color is removed from them.

Figure 5 is a drawing of a portion of a yellow filament of *Clonothrix*, more highly magnified and showing the detail of the sheath. A darker portion (brownish in color) lies just within a lighter, more transparent layer on the surface. This was unstained material, mounted in glycerine, so that it was possible to complete the study before any change had taken place in color due to the mounting medium. In his new species, *Leptothrix crassa*, Cholodny (1924) finds the sheath differing from the classical *L. ochracea* in that the former has an uneven surface and the latter a smooth one, and that while the former is granular and less refractive, the latter is transparent and highly refractive. He suggests (1936) that Molisch (1910) and Lieske (1919) were working with his *Leptothrix crassa* in their physiological experiments instead of *L. ochracea*. The relation of the structure of the sheath to iron or manganese accumulation by these organisms is an important point for further study. Although my study is insufficient to offer support or

criticism of any of the theories advanced in regard to this (Winogradsky, 1888, 1922; Molisch, 1910; Ellis, 1907, 1910; Lieske, 1919), two points might be worth mentioning. I find that if the filaments of *Clonothrix fusca* are kept in tubes of sterile water over a period of six months, they still retain their brown color, but the cells have disappeared from the sheaths and from the tips. My figure 5 brings out the second point. Within the thicker outer sheath there is an inner colorless sheath which surrounds the cells and which projects, when the filament is broken, beyond the yellow-brown sheath. Roze also figured the emerging of an inner filament of eight cells from a broken sheath. Frequently, however, individual cells emerge independently from the broken ends of old filaments. I observed the escape of such cells from the sheath in filaments which had been mounted in water and accidentally allowed to dry. Such slides present the appearance of a great many fragments of filaments. When water was again added to the mount, I observed five cells successively emerge from within one sheath and each one leave the immediate vicinity of the filament. I could not determine any means of locomotion, and the slowness of their release seemed rather to indicate they had been gently forced out, perhaps by a swelling of mucilage within the sheath. It is not unusual to observe cells projecting from the sheath when the filament is broken, but I observed actual escape of successive cells only once. Escape of cells from the sheath has been reported for *Leptothrix* by Molisch, *Cladothrix* by Zopf, and for *Clonothrix* by Roze, West, and Schorler.

The cells within the sheaths of *Clonothrix fusca* are colorless, although an almost imperceptible greenish tinge was observed by me in one instance in the cells at the tip of a filament. They are cylindrical with rounded ends, the variation in length covering a wider range than that in width. The average length is $10\ \mu$, the average width, $2\ \mu$. I have found cells as long as $19\ \mu$, and on one occasion one $47\ \mu$ long, but the width of these cells was average. Very long cells are frequently found at the tips of the filaments, and these divide to form spores. These long cells frequently show evidence of division (fig. 1, 2, 3). At the tips of the long branches (fig. 9) the cells are sometimes as narrow as $1.6\ \mu$, but those in the oldest portion of the filament are not wider than $2.4\ \mu$, the decrease being very gradual from base to apex. Abrupt decrease in size was noted only in the short, whorled branches (mentioned above) composed of five or six cells; but these branches had apparently ceased active growth, since they were enclosed in a yellow sheath. Roze gives the measurements of his *Clonothrix* filaments about seven micra in diameter in the brownish or mature portions, about $5\ \mu$ in the yellowish portions, and $3\ \mu$ in the young, almost transparent or slightly bluish portions at the tips. Schorler describes young filaments 2 – $3\ \mu$ thick and old ones 5 – $7\ \mu$ thick, narrowing to $2\ \mu$ at the tip. He claims to have found old filaments with an accumulation of manganese in the sheath as wide as $24\ \mu$,



but I have observed no filaments as wide as this. He states that the cells are 2μ thick and usually $6-8\mu$ long. Both Roze's and Schorler's measurements of the filaments (sheath plus cell width) correspond to my measurements of living, unstained filaments (given above).

In contrast to the long, cylindrical cells of *Clonothrix*, the *Crenothrix* filaments which I have studied (fig. 8) have an average cell length of 2.8μ , ranging from $1.2\mu-5.2\mu$, and an average width of 2.0μ ranging from $1.2\mu-2.4\mu$. The cells are rarely more than twice as long as broad, and the colorless sheaths do not add appreciably to the width of the filament. The filament of *Crenothrix* presents a very different appearance with the smaller, tapering cells at the base than that of *Clonothrix* with its tapering apical end (compare fig. 8, 9).

When spore formation takes place, numbers of short branches arise from the colorless portions of the *Clonothrix* filaments, and the cylindrical cells which compose them rapidly divide and redivide in one plane, so that chains of spherical spores are formed, which are borne on the sides of the filaments, giving them a racemose appearance near the tips (fig. 6), and there is evidence of bi-partition of the cylindrical cells to form the spores. A continuous sheath surrounds the spores of each branch and is in contact with the side of the filament from which the branch originated. It is colorless and assumes the contour of the individual spores, around which it fits so closely that it is frequently invisible, except at higher magnification than that of figure 6, or when the material is specially stained. Figure 4, stained with polychrome methylene blue, shows this, as does figure 1, stained with aceto-carmin. In figure 1, the older spores are nearest the base of the branch, and the sheath has assumed the contour of the individual spores with constrictions between them. Above these, nearer the tip, are spores arranged in pairs, indicating that division has been only recently completed, and the sheath does not yet show the constrictions between them. At the tip are two cylindrical cells which have not yet divided. There is an indication here that spore formation is acropetal, the younger spores being at the tips of the filaments. Two of the spores have

slipped out of position within the sheath, so that they come to lie one slightly behind the other. This appearance is unusual for my *Clonothrix* material, although it is common in *Crenothrix* (fig. 8b). In *Clonothrix* the spores are usually arranged linearly in a single row, due to the fact that the cylindrical cells divide only in one plane at right angles to the long axis of the filament. In *Crenothrix*, however, spore formation is accomplished by cell division in a number of planes, as the portion *a* in the filament of *Crenothrix* in figure 8 shows—i.e., bipartition of the cells is taking place longitudinally, transversely, and obliquely. After rounding up, the spores are arranged more or less regularly in two rows alternate with each other (fig. 8b). At the tip they lie in a single row, as they are released from the filament. This is not always the case, since I have also observed the filament gradually widen toward the tip to accommodate several rows of spores which are then released in a mass, as deBary's (1884) reproduction of Zopf's figure illustrates. In *Crenothrix polyspora* the spores mature in basipetal succession, the youngest spores being farthest removed from the tip of the unbranched filament.

The tendency of the spores of *Clonothrix* to be formed in short branches near the tips of the filaments was also noted by Schorler, as was also the presence of the surrounding sheath; but he describes the cells of the filament as dividing parallel to its long axis, whereas, in my material, the cells divide transversely to the long axis of both the filament and of the cell. This difference may be due to the shape of the cells which give rise to the spores, since he describes flat, disk-shaped cells as dividing to form spores, whereas my cells were long and cylindrical, like the ordinary vegetative cells of the filament. I do not find any of these flat, disk-shaped cells mentioned by Schorler. The size of his spores (1.5μ) is smaller than I find in my material, the average size of 45 spores ranging from $1.6\mu-3.2\mu$ being 2.24μ . My *Crenothrix* spores were smaller, averaging 1.6μ in diameter. The presence of macro- and microgonidia have been reported in a number of the iron bacteria. Although I have not observed the release of the spores from their sheath, I have noted the

Fig. 1-9.—Fig. 1. *Clonothrix fusca*. Tip of filament with branch showing beginning spore formation inside the sheath; oldest spores at base of branch. 1600X. Aceto-carmin stain.—Fig. 2. *Clonothrix fusca*. Tip of filament showing spore branches; continuation of upper end of filament represented in fig. 9. 800X. Mounted in glycerine, unstained.—Fig. 3. *C. fusca*. Tip of filament showing beginning of a false branch at *a*. At points marked *x*, after an interval of time, the cells separated, showing the sheath, as indicated above each of these points. Otherwise, sheath invisible around vegetative cells and spore branch. Living material, unstained. 800X.—Fig. 4. *C. fusca*. Portion from tip of filament observed under oil immersion objective. Sheath around spores distinctly visible, and constrictions evident between spores. Polychrome methylene blue stain. 1600X.—Fig. 5. *C. fusca*. Older portion of a filament showing an inner filament emerging from the broken end of the brownish yellow sheath. 1600X. Mounted in glycerine, unstained.—Fig. 6. *C. fusca*. Four portions from a single filament; *a*, the oldest portion where the sheath is brownish yellow; *b* and *c*, portions nearer the tip with respectively thinner sheaths; *d*, tip of the filament where the sheath is difficult to see and where the spore branches present a racemose appearance. Aceto-carmin stain. 800X.—Fig. 7. *C. fusca*. Portion of a filament showing branching. 1600X. Polychrome methylene blue stain.—Fig. 8. *Crenothrix polyspora*. Entire filament with portion omitted between dotted lines, but showing base and apex of the filament. At *a* cells have divided but have not yet rounded up to form the spores. At *b*, the rounded spores lie in two rows, alternating with one another. Aceto carmin stain. 800X.—Fig. 9. *Clonothrix fusca*. A long filament with portions omitted as indicated, showing arrangement of branches alternate and opposite. Unstained, mounted in glycerine. 800X.

sheaths to be open at the tips of the branches (fig. 4 for *Clonothrix*; fig. 8 for *Crenothrix*). I have also observed large gelatinous masses of freed spores, bringing to mind the zoogloea described by Zopf (1882) for *Cladothrix dichotoma*. Except for its unique spore branches, *Clonothrix fusca* shows a close resemblance to *Cladothrix dichotoma*.

The cells, which I have described as spores, come under the type called arthrospores (deBary, 1884, page 496) and have frequently been called gonidia in the literature. They are not endospores in the meaning of the bacteriologists, where, as in *Bacillus anthrax* and *B. subtilis*, a spore is formed by a massing of the protoplasm in a certain place inside the cell membrane. They are, however, endospores in the sense of the term used by Smith (1933) for the blue-green algae, who describes them as formed by repeated division of the protoplast within the cell wall. The sheath is considered a part of the cell wall. I have preferred to use the general term "spore" since "arthrospore" is hardly more specific.

SUMMARY

The following differences between *Clonothrix fusca* Roze and *Crenothrix polyspora* Cohn may be enumerated:

The general habit of *Clonothrix* is a branched filament—the type designated as false branching in *Cladothrix dichotoma* by Cohn and also found in many of the blue-green algae. *Crenothrix* is an unbranched filament.

The *Clonothrix* filament tapers to the tip, and with gradual accumulation of ferric hydroxide in the sheath, it becomes wider in the older portions, but this increase is not only accumulation on the surface, since with removal of the brown substance from the sheath there is evidence that the sheath itself is thicker in

the older portions. The *Crenothrix* filament may taper to the apex or become broader according to the arrangement of spores in one or more rows inside the sheath, but it invariably tapers to the base or point of attachment, where the cells also are smaller.

The sheath of *Clonothrix* is thin and colorless in the young apical portions and is difficult to see, but it becomes yellowish brown, thick, and brittle in older portions. The sheath of *Crenothrix* in my material is colorless throughout its entire length, and although not thick, is distinctly visible at both apex and base of the filament.

The cells of *Clonothrix* are colorless and are cylindrical in shape with rounded ends, usually at least 5 times as long as wide. The cells of *Crenothrix* are also colorless, and although about the same average width, are usually not more than twice as long as wide.

Spore formation in *Clonothrix* is acropetal and seems to be limited to short branches at the younger portions of the filaments. Spore formation in *Crenothrix* is basipetal, and practically all cells of the entire filament may become spores.

Spore formation in *Clonothrix* follows successive transverse bipartitions of the long cylindrical cells in one plane, giving rise to single chains of spores. Spore formation in *Crenothrix* follows cell division by bipartition in more than one plane and may take place transversely, longitudinally, and obliquely, resulting in smaller spores than those of *Clonothrix*.

The spores of *Clonothrix* are surrounded by a sheath which becomes constricted between spores. The sheath enclosing the *Crenothrix* spores remains tubular and does not show these constrictions.

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THE INFLUENCE OF ENVIRONMENTAL CONDITIONS AT PLANTING TIME ON SORGHUM KERNEL SMUT INFECTION¹

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BREEDING FOR RESISTANCE to sorghum kernel smut, *Sphacelotheca sorghi* (Link) Clint., requires a knowledge of the reaction of varieties, parental material, and hybrids to the disease. For investigations of the inheritance of resistance to sorghum kernel smut, it is imperative that plantings are made at times when maximum infection is obtained in susceptible plants. Studies on varietal resistance and the inheritance of resistance to sorghum kernel smut, *S. sorghi*, have been in progress at Manhattan, Kansas, for 15 years. During this period there have been seasons when satisfactory records on *S. sorghi* infection were not obtained because of failure to secure heavy infection. The results of this paper should aid in determining the optimum planting dates and other conditions necessary for maximum sorghum kernel smut infection.

The relationship between the date of planting sorghum and infection by the kernel smut *Sphacelotheca sorghi* under field conditions has not been studied extensively. The authors have obtained data in the field which indicate that soil temperature and soil moisture largely determine the amount of smut infection that may occur when the seed is naturally or artificially smutted. Weekly date of planting experiments were conducted at Manhattan, Kansas, from 1932 to 1936, inclusive. The plantings were made during the period of March to July, inclusive, to determine (1) the relationship between environmental factors and infection of sorghum² by *S. sorghi*, (2) the dates and under what soil conditions sorghum should be planted to obtain maximum infection, and (3) whether sorghum plantings can be made so as to avoid infection and yet secure normal growth and mature grain.

REVIEW OF LITERATURE.—Kulkarni (1922) suggested that soil temperature was an important factor in producing infection in sorghums by *Spacelotheca sorghi*.

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² In the use of the word sorghum in this paper the writers have reference to Blackhull kafir or Pink kafir which were the only varieties used in the experiments.

He observed that under field conditions no infection occurred at Jacobabad, India, where the temperature at the time of seeding was from 36°-40°C., while 65 per cent infection occurred at Poona during a corresponding period, but with a temperature of 25°C. at planting. He also found that the optimum temperature for the germination of sorghum seed was 36°-40°C., while very few spores of the smut germinated at that temperature. The optimum temperature for the germination of the spores was 20°-23°C., and at this temperature the sorghum seed required one to two days longer to germinate. He concluded that temperature was the limiting factor in the distribution of *S. sorghi* in India. Kulkarni does not state whether the temperatures (36°-40°C.) are soil or air temperatures. According to studies made at Manhattan, Kansas, a temperature of 40°C. in a seed germinator is not a satisfactory temperature for sorghum seed germination. We find 27°C. is an optimum temperature, although good results are obtained at temperatures of 20° to 30°C.

Reed and Faris (1924a, 1924b) conducted investigations on the effect of environmental factors on the infection of sorghums with *S. sorghi* and *S. cruenta*, the covered and loose kernel smuts of sorghum, under controlled conditions in temperature tanks in the greenhouse. They found that soil moisture, soil temperature, and soil reaction were interdependent factors determining the severity of infection, and that any one of the factors may be the limiting one in the prevention of infection. Therefore, the optimum condition for any one of the three would be without significance unless due regard were paid to the other possible influencing factors. They observed that infection occurred in susceptible varieties over a wide range of temperatures. In every case infections were secured at 15°C. Infection occurred over a wide range of soil moistures with particularly high infections in the lower soil moistures. A rather wide range of soil reactions produced infection, slightly acid soils being the most favorable for high infections. The optimum temperature for infection of sorghum by *S. sorghi* varied with different varieties. Darso gave

the highest infection at 20°C., Blackhull kafir at 15°C., and Red Amber sorgo at 25°C. The optimum soil moisture for infection did not vary so much for the different varieties as did the temperature. Blackhull kafir and Valley kaoliang gave the highest infection when the soil contained 10 per cent of its water-holding capacity, although the amount of infection was cut down considerably at the higher moistures.

Vinall and Reed (1918), in a study of the effect of meteorological factors on sorghums, found that to secure the maximum yield and other desirable agronomic qualities, dates of planting should be so arranged that germination and early growth of the plants would take place during the period of high temperatures, and the flowering and fruiting when more moderate temperatures prevail.

Martin, Sieglinger, and associates (1929) found that the optimum date for seeding grain sorghums varied for different varieties. In general the earliness of seeding should be inversely proportional to the earliness of maturity. Medium-late seeding resulted in better stands, taller stalks, larger heads, and shorter periods than did the early seeding. Certain groups of sorghums, such as the milos and feteritas, were very sensitive to low temperatures at seeding time and ordinarily should not be sown until the soil is warm. In the southern Great Plains the late varieties should be planted about May 15, while the early varieties should be planted about June 15.

Laude and Swanson (1933) also found that sorghums should be planted so as to avoid low temperatures during germination, extremely high temperatures during the blooming and filling periods, and to provide ample time to ripen before the first killing frost in the fall. In an experiment conducted at Fort Hays Agricultural Experiment Station from 1925-1931, inclusive, it was found that in general the best grain yield of the late varieties was obtained by planting about May 15 and of the early varieties about June 15.

MATERIALS AND METHODS.—Blackhull kafir C.I. 71 was used during 1932, 1933, and 1934, and Pink kafir C.I. 432 during 1935 and 1936. The latter variety matures earlier and was therefore thought to be better suited for these studies. Both varieties, however, are very susceptible to kernel smut.

The inoculum consisted of *S. sorghi* physiologic race 1 and was collected from the previous year's crop in the section of the nursery set aside for the increase of physiologic races (Melchers, Ficke and Johnston, 1932). Smutted heads were ground in a meat grinder and the mass was screened to free it from debris. A quantity of seed sufficient to plant each year's experiment was inoculated by adding the chlamydo-spores to the seed in a quart jar and shaking it vigorously to give a uniform distribution of spores. The surplus spores were removed by screening the seed.

A series of periodic plantings were made, starting shortly after the middle of March and continuing

until the latter part of July. Each planting consisted of a 50-foot row of the inoculated seed sown by hand. The seed was covered to a depth of two to three inches in a uniform, well-tilled seedbed. Two soil samples were taken at the depth of planting in different places in the furrow and their moisture determined on a dry basis. The average of these was used for that particular date of planting.

The soil temperatures were obtained from the charts of the soil-air thermograph which was located near the plots. The average minimum soil temperature refers to an average of the minimum temperature each day from the date of planting to the date of emergence of the seedlings. The average maximum temperature for each planting was similarly calculated for the maximum temperatures of the same period. The absolute maximum and minimum temperatures represent the highest and lowest temperatures recorded during each germination period and also are given as a matter of reference.

The rainfall is recorded by dates, and the total amount that fell from one date of planting until the next is also indicated in the tables.

A number of soil samples were taken from the plot and tested for acidity. The samples ranged from pH 6.2 to 6.8 with an average of 6.5. As this is approximately the optimum soil reaction for sorghum-kernel-smut infection, no further consideration was given this factor.

When the plants were four to six inches tall, they were thinned to about eight inches apart in the row. Date of emergence notes were taken when approximately 50 per cent of the seedlings were showing the coleoptiles. No emergence notes were taken during 1932 and 1933; therefore, the dates of emergence were estimated by using data obtained in 1934-1936. In this way 21 days were allowed for the planting made in March, 17 days for the first planting in April, 14 days for the second planting in April, 10 days for the next 3 plantings, and seven days from then on.

The total number of plants and the number of smutted plants in each row were recorded at the end of the season. The percentage of smut was based on the number of smutted plants and not smutted tillers in each row. In certain years the July sowings did not have sufficient time to produce heads; therefore, smut infection notes could not be secured.

RESULTS.—The graphs illustrate the results obtained in these studies. It is apparent that variations occur in the soil temperature and soil moisture during the time that infection may have taken place for each planting. Certain limitations must be placed, therefore, on the relationship between the average maximum and average minimum soil temperatures, the average soil moisture at planting, and the percentage of smut infection that occurred. Natural variability also may account for some variation.

In 1932 a moderate amount of infection occurred for each planting except those made on June 13 and July 4 (table 1, fig. 1). The weekly plantings for this year, however, were not continued long enough

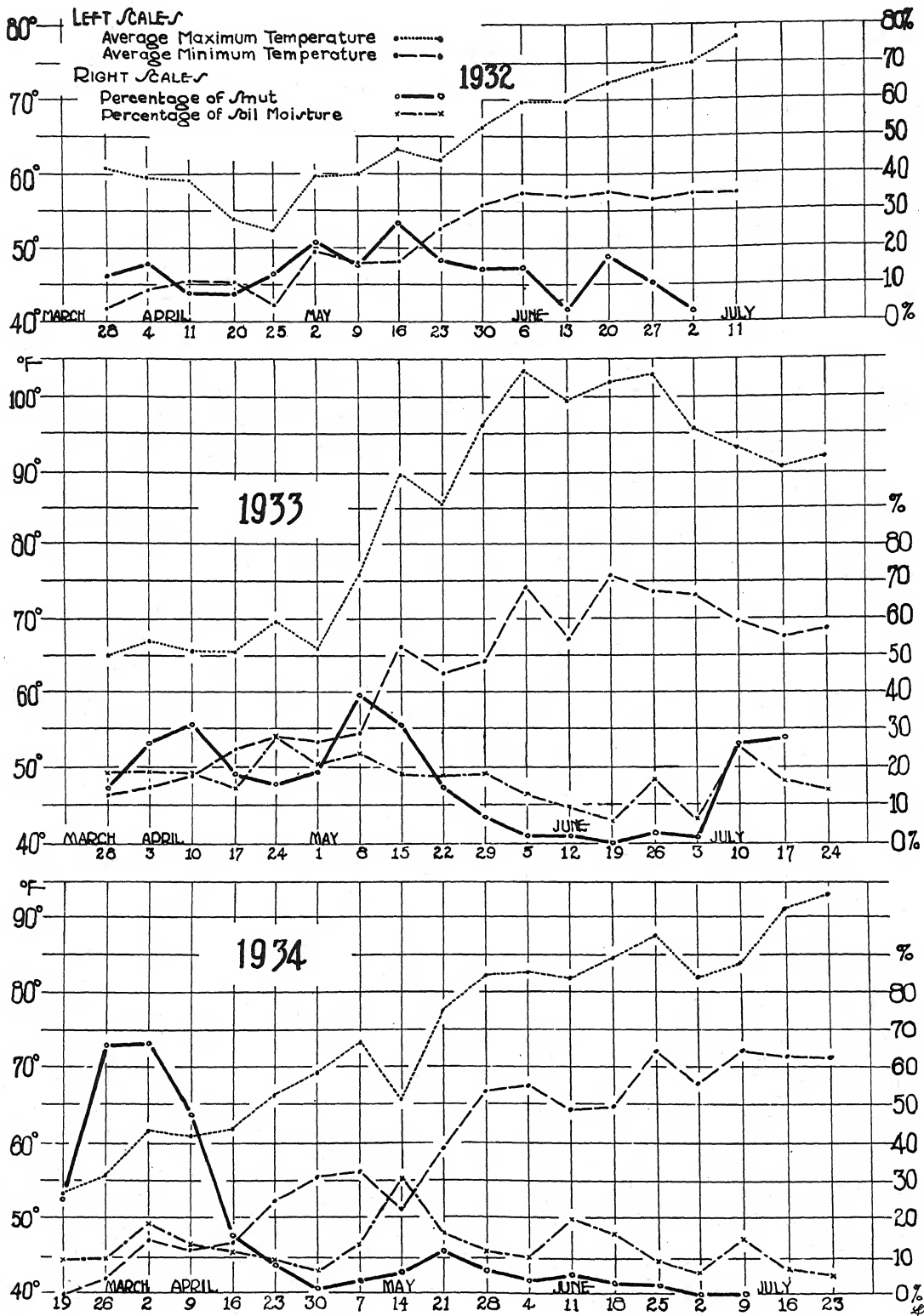


Figure 1.

TABLE 1. *The relation of soil temperature and moisture to sorghum kernel smut infection in experimental field plots at Manhattan, Kansas, 1932.*

Date sown	Date of emergence ^a	Total number plants	Smut percentage	Soil temperature, °F.				Daily rainfall in inches ^b	Total rainfall
				Minimum		Maximum			
				Mean	Absolute	Mean	Absolute		
March 28	April 18	117	12.0	41.8	28	60.9	68	None	
April 4	April 21	101	15.8	44.2	33	59.9	68	April 7 (.01)	.01
April 11	April 25	94	7.4	45.2	33	59.2	68	April 16 (.08); 17 (.12); 19 (.22)	.42
April 20 ^c	April 30	100	7.0	45.1	30	53.8	68	April 20 (1.00); 21 (.07); 23 (.12); 24 (.17)	1.36
April 25	May 5	119	12.6	42.2	30	52.2	66	April 25 (.10); 28 (.02)	.12
May 2	May 12	102	21.6	49.4	41	59.9	66	May 3 (.09); 6 (.03); 7 (.67); 8 (.09)	.88
May 9	May 16	112	16.1	47.8	41	60.0	65	May 9 (.36)	.36
May 16	May 23	127	26.8	48.0	42	63.1	70	May 16 (.05)	.05
May 23	May 30	124	16.1	52.7	44	62.0	70	May 26 (.81); 29 (.27)	1.08
May 30	June 6	100	14.0	55.8	44	66.1	71	May 31 (.30); June 3 (.73)	1.03
June 6	June 13	125	14.4	57.4	55	69.6	74	June 12 (.20)	.20
June 13	June 20	93	3.2	56.7	52	69.8	76	June 13 (.80); 19 (.94)	1.74
June 20	June 27	52	17.3	57.6	52	71.7	76	June 20 (1.44); 26 (.14)	1.58
June 27	July 4	67	10.4	56.3	51	73.7	80	June 27 (.63)	.63
July 4	July 11	107	2.8	57.3	54	75.0	83	July 5 (.71); 8 (.01); 9 (.18)	.90
July 11 ^d	July 18	110	—	57.3	55	78.7	80	None	

^a Approximate.^b Precipitations of less than .01 inch are not included.^c Unavoidable circumstances prevented the weekly planting on April 18.^d The plants did not head before frost.

TABLE 3. *The relation of soil temperature and moisture to sorghum kernel smut infection in experimental field plots at Manhattan, Kansas, 1934.*

Date sown	Date of emergence	Total number plants	Smut percentage	Soil temperature, °F.				Soil moisture percentage at planting	Daily rainfall in inches ^a	Total rainfall
				Minimum		Maximum				
				Mean	Absolute	Mean	Absolute			
March 19	April 9	85	22.4	39.9	31	53.2	70	8.8	Mar. 24 (.01)	.01
March 26	April 9	99	65.6	41.7	31	55.4	70	9.5	Mar. 30 (.28); Apr. 1 (.33)	.61
April 2	April 11	94	66.0	47.2	41	61.6	70	18.2	Apr. 4 (.06)	.06
April 9	April 20	89	47.2	45.7	40	60.9	70	12.8	Apr. 15 (.10)	.10
April 16	April 26	97	15.5	46.5	40	61.9	66	10.9	None	
April 23	May 8	92	7.6	52.1	45	66.4	80	9.0	Apr. 26 (.03)	.03
April 30	May 11	86	1.2	55.2	51	69.4	80	6.6	May 4 (.49); 5 (.42)	.91
May 7	May 13	84	3.6	55.8	50	73.1	80	13.2	May 10 (.71); 13 (2.23)	2.94
May 14	May 19	79	5.1	51.0	47	65.7	76	30.5	May 14 (.32)	.32
May 21	May 26	86	11.6	59.2	55	77.5	80	15.6	None	
May 28	June 6	64	6.2	66.7	61	82.0	86	11.8	None	
June 4	June 12	84	3.6	67.3	61	82.7	86	10.1	June 10 (.53)	.53
June 11	June 15	82	4.9	64.4	61	81.6	88	19.6	June 11 (.15); 14 (.35); 15 (.50); 16 (.12); 17 (.02)	1.14
June 18	June 22	77	2.6	64.8	59	84.4	86	16.3	June 22 (.22)	.22
June 25	June 29	51	2.0	72.2	72	87.2	88	8.8	None	
July 2	July 10	60	0.0	68.0	59	81.6	87	5.2	July 6 (.61)	.61
July 9	July 17	7	0.0	72.2	69	83.8	87	14.5	None	
July 16	Aug. 3	^b		71.4	65	91.2	96	6.6	None	
July 23	Aug. 8	^b		71.2	65	92.9	100	5.1	July 26 (.08); 27 (.16)	.24

^a Precipitations of less than .01 inch are not included.^b Development checked by heavy frost.

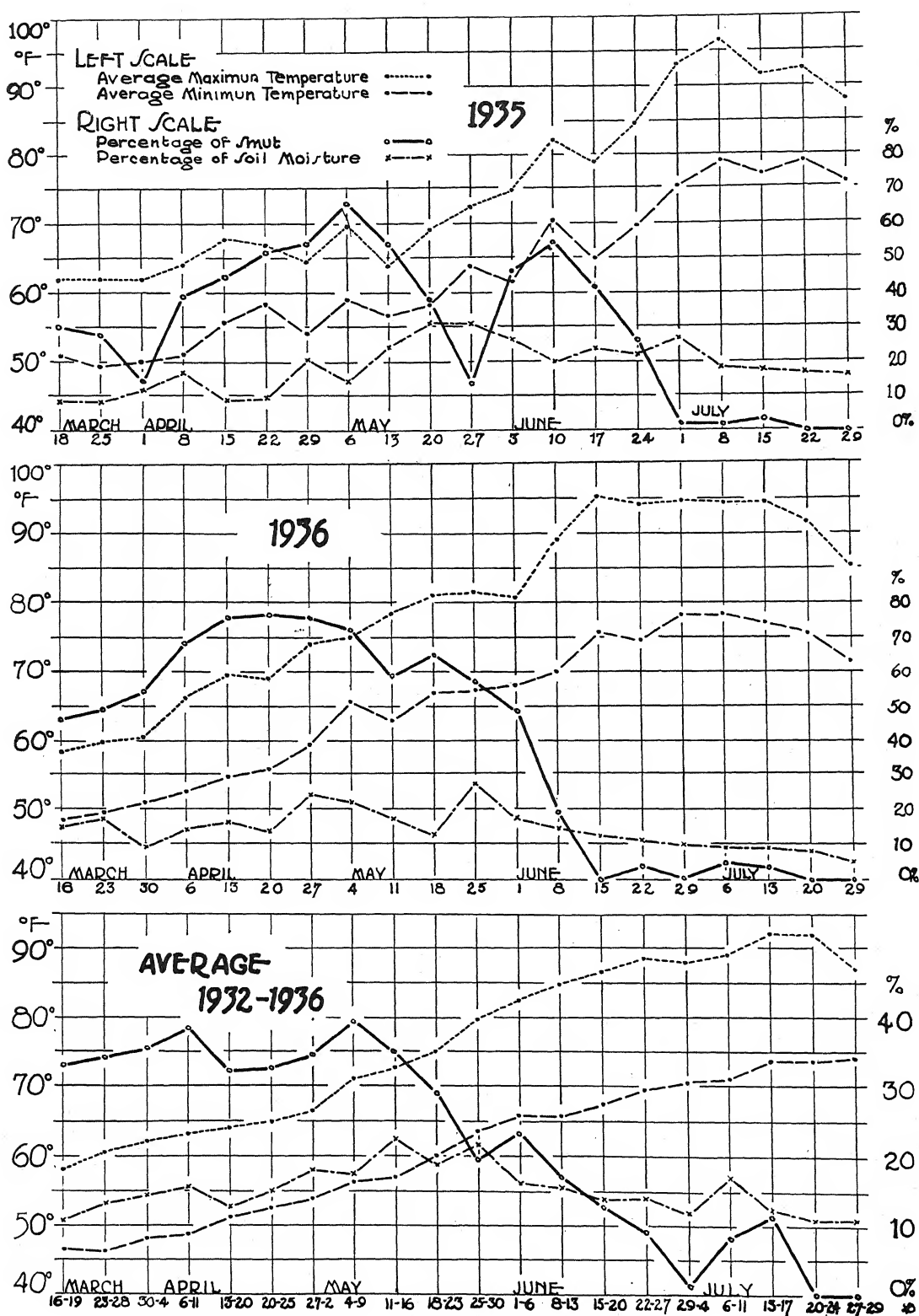


Figure 2.

TABLE 4. *The relation of soil temperature and moisture to sorghum kernel smut infection in experimental field plots at Manhattan, Kansas, 1935.*

Date sown	Date of emergence	Total number plants	Smut percentage	Soil temperature, °F.				Soil moisture percentage at planting	Daily rainfall in inches ^a	Total rainfall
				Minimum		Maximum				
				Mean	Absolute	Mean	Absolute			
March 18	April 8	54	29.6	51.0	44	61.9	72	8.0	None	
March 25	April 22	69	27.5	49.7	44	61.9	74	8.0	None	
April 1	April 24	52	13.5	49.9	44	61.8	74	10.8	Apr. 7 (.29)	.29
April 8	April 24	66	39.4	51.0	44	64.2	74	16.9	Apr. 10 (.05)	.05
April 15	April 26	78	44.9	55.4	46	67.9	74	8.5	Apr. 18 (.04)	.04
April 22	May 2	77	50.6	58.7	50	67.1	72	9.2	Apr. 25 (.03); 28 (.20)	.23
April 29	May 9	61	54.1	54.1	49	64.4	71	20.9	Apr. 29 (.44); May 3 (.11)	.55
May 6	May 13	76	65.8	59.2	55	70.0	74	13.2	May 9 (.27); 11 (.93); 12 (.12)	1.32
May 13	May 26	74	54.1	56.8	54	64.3	76	23.8	May 13 (.07); 14 (1.05); 16 (.11); 18 (.64); 19 (.96)	2.83
May 20	May 28	63	38.1	53.8	50	69.2	76	30.7	May 20 (.42); 21 (.02); 23 (.05); 26 (.02)	.51
May 27	June 4	74	13.5	64.0	61	72.6	76	30.4	May 27 (.65); 28 (1.67); 31 (.53); June 1 (.53); 2 (.54)	3.92
June 3	June 10	77	48.1	61.4	55	74.5	85	26.0	June 3 (.01); 6 (.15)	.16
June 10	June 17	71	54.9	70.5	67	82.0	86	19.7	June 11 (.36); 12 (.30); 13 (.18); 14 (.06)	.90
June 17	June 23	70	41.4	64.8	59	79.1	85	23.2	June 17 (.38); 18 (.01); 19 (.01); 21 (.28)	.68
June 24	June 29	65	26.2	69.7	68	84.5	86	21.4	June 25 (.64); 26 (2.04); 28 (.75); 29 (.11); 30 (.44)	3.98
July 1	July 6	67	1.5	75.2	72	93.3	98	26.4	July 4 (.02)	.02
July 8	July 13	65	1.5	78.2	74	96.5	100	18.6	None	
July 15	July 21	41	2.4	77.0	75	91.6	94	17.6	None	
July 22	July 28	35 ^b	0.0	79.0	77	92.4	94	17.0	July 24 (.02)	.02
July 29	Aug. 23	31 ^b	0.0	76.2	68	88.5	97	16.5	Aug. 3 (.03); 10 (.13); 18 (.39); 19 (.20); 20 (.31); 21 (2.05)	3.11

^a Precipitations of less than .01 inch are not included.^b Less than 50 per cent of plants headed.

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into July to determine the effect of very high temperatures on smut infection. No soil moisture determinations were made in 1932.

In 1933 a moderate to fairly high infection occurred in the first nine and in the July 10 and 17 plantings (table 2, fig. 1). High soil temperatures during June and the first part of July reduced the infection markedly. It would appear as if high soil moisture may have helped to reduce the amount of infection for the April 24 planting. The high smut infection in the July 10 and 17 plantings appears to be associated with the drop of the maximum temperatures on these dates. The soil moisture was favorable for infection.

In 1934 medium to high infections occurred in the plantings during March and up to April 16, while very low to no infection occurred during the remainder of the plantings in April, May, June, and July (table 3, fig. 1). In general, it is apparent that as the soil temperatures rose, smut infection decreased. This same tendency was apparent in 1933. Some other unknown factor, however, besides soil temperature and soil moisture must have had a part in reducing the infection during late April and May plantings. The drought of 1934 was so severe that unusual conditions entered into the experiment.

It was necessary to irrigate the plants in certain rows when they were about half grown. This was done on three occasions; otherwise, the plants would have died from the drought. The plants at the ends of these rows were just heading when the water was applied, while those in the center of the rows were two to three weeks behind in their development and not headed.

It was thought advisable to observe the influence of irrigation on the development of smut in the unheaded plants. Counts were made separately on the number of smutted heads that occurred on plants in the ends and center of the rows. A tabulation showed, however, that no significant difference was found in the percentage of smut in the two parts of the rows.

The results in 1935 show that low soil temperatures during the first plantings reduced the infection slightly; however, the percentages of infection are by no means low, while the very high temperatures in July reduced the infection to less than three per cent (table 4, fig. 2). A rainy period during the latter part of May seems to account for the drop in infection during that period, since the precipitation was heavier than usual and the soil was actually wet. Although the temperature was satisfactory for infection, it appears that the soil moisture was a limiting factor in this case. During the next two plantings, as the soil moisture decreased, smut infection rose rapidly, but dropped in the July plantings, as the soil temperature rose to the inhibiting point. The relation between the high soil temperatures and the lack of infection, even though the soil moisture was favorable, was apparent in 1935. These results are very comparable to those of Reed and Faris (1924a, 1924b) under controlled conditions in the greenhouse.

The effect of temperature on infection in Pink kafir in 1936 was very similar to that of 1935 (table 5, fig. 2). The mean minimum soil temperatures for the first three dates of planting in March did not inhibit high percentages of infection. The April and early May plantings produced still higher infection, as very favorable soil temperature and moisture conditions prevailed. The high soil temperatures after June 15 reduced the infection to less than six per cent as was the case in 1935. The soil moisture remained fairly favorable during the greater part of the season except during the plantings in late June and July when the soil moisture was too low for the germination of the kafir seed. High soil temperatures prevailed, and it was several weeks before the seed germinated. These plantings gave little or no infection. The maximum soil temperatures, together with the very high absolute maximums, appear in themselves to have been high enough during the germinating period to be the limiting factor in smut infection. This is shown in the June 15 to July 27 plantings.

The average results during the five years indicate that in general there was a slight increase in the percentage of infection up to the first week in May and then a fairly rapid decrease until the latter part of June. The infection in July was low, varying from 11.2 per cent to none at all (table 6, fig. 2).

The soil temperature was the most important single factor in determining sorghum kernel smut infection, as the pH determinations indicated that the soil acidity in the plots could not be a limiting factor. Except in a few cases when the soil was very wet, the soil moisture did not affect the amount of infection, provided the seed germinated and the plants grew to the heading stage.

The foregoing data seem to indicate that in order to obtain very high infection with *Sphacelotheca sorghi* for experimental purposes, it is best not to plant later than May 15. They also suggest that under conditions comparable to those at Manhattan, Kansas, the highest infection can be expected in sowings made during the latter part of April or the first few days in May. Where losses from frost damage is of secondary importance and high smut infection the primary consideration, sowings made during the first week in May could be expected to give the most satisfactory results and to be reasonably safe.

A fairly high infection may be expected, therefore, on any date that it is practical to plant sorghum. These studies show that sorghum smut cannot be avoided in farm practice by changing the planting date. In commercial practice, naturally smutted seed must be treated by one of the standard fungicides.

SUMMARY

Plantings of sorghums were made at weekly intervals over a period of five crop years at Manhattan, Kansas, to determine the relationship of environmental factors to *Sphacelotheca sorghi* infection in the field.

TABLE 5. *The relation of soil temperature and moisture to sorghum kernel smut infection in experimental field plots at Manhattan, Kansas, 1936.*

Date sown	Date of emergence	Total number plants	Smut percentage	Soil temperature, °F.				Soil moisture percent- age at planting	Daily rainfall in inches ^a	Total rainfall
				Minimum		Maximum				
				Mean	Absolute	Mean	Absolute			
March 16	April 13	28	46.4	48.7	45	58.4	71	15.1	Mar. 16 (.19)	.19
March 23	April 14	51	49.0	49.6	46	59.8	76	16.8	None	
March 30	April 16	79	54.4	50.7	46	60.4	76	9.1	Apr. 1 (.03); 2 (.07)	.10
April 6	April 18	81	67.9	52.6	47	66.2	76	13.9	Apr. 7 (.01); 10 (.17)	.18
April 13	April 23	81	75.3	54.4	49	69.4	76	16.6	None	
April 20	April 29	86	76.7	55.4	49	69.0	77	13.4	Apr. 21 (.75); 26 (.07)	.82
April 27	May 4	85	76.5	59.4	55	74.1	83	23.9	Apr. 27 (.18); 28 (.09); 29 (.03); May 1 (1.86); 2 (.01)	2.17
May 4	May 10	73	72.6	65.4	61	75.3	82	21.2	May 8 (.27); 9 (.44); 10 (.08)	.79
May 11	May 18	68	58.8	63.1	58	78.4	86	17.2	May 13 (.17)	.17
May 18	May 24	77	64.9	66.8	63	81.1	87	12.6	May 23 (.08); 24 (2.72)	2.80
May 25	June 1	39	56.4	67.2	65	81.5	84	27.0	May 25 (.15)	.15
June 1	June 8	31	48.4	68.0	66	80.5	91	16.6	June 5 (.12); 6 (.45)	.57
June 8	June 14	58	19.0	70.0	67	89.3	94	13.9	None	
June 15	June 21	21	0.0	75.4	72	95.3	98	11.8	June 17 (.18)	.18
June 22	June 28	24	4.2	74.6	70	94.4	100	10.5	None	
June 29	Aug. 4	29	0.0	77.9	75	94.8	99	9.5	None	
July 6	Aug. 4	36	5.6	77.9	66	94.4	104	9.2	None	
July 13	Aug. 3	29	3.4	77.0	66	94.6	104	9.1	None	
July 20	Aug. 3	37	0.0	75.4	66	91.4	104	7.4	None	
July 27	Aug. 2	40	0.0	71.4	66	85.3	104	5.1	July 28 (1.78)	1.78

^a Precipitations of less than .01 inch are not included.

In the vicinity of Manhattan, Kansas, varietal resistance or susceptibility to sorghum kernel smut can best be determined by planting from 10 to 14 days earlier than is recommended for farm practice. There is less danger of failure to obtain satisfactory smut infections in plantings prior to May 15 than after that date.

Sorghum kernel smut cannot be escaped by early planting. In the vicinity of Manhattan, Kansas, ordinarily, plantings as late as July may escape kernel smut, but early frosts do not allow the crop to mature normally.

In general, when the mean maximum soil temperatures during the infection period are 75°F. or above, the amount of infection is reduced. It must be remembered, however, that during the germination and infection periods, it may be possible that one or two high temperatures may inhibit infection, even though the average of the maximum temperatures does not appear adverse to infection under field conditions.

Soil moistures of 28 per cent or more on the dry basis, even though favorable soil temperatures prevail, reduce the infection considerably.

TABLE 6. *The relation of soil temperature and moisture to sorghum kernel smut infection in experimental field plots at Manhattan, Kansas. Averages of 5 years' results, 1933-1936.*

Date sown	Mean temperature		Average smut percentage	Average soil moisture percentage
	Minimum	Maximum		
March 16-19	46.5	57.8	32.8	10.6
March 23-28	45.9	60.6	33.7	13.2
March 30-April 4	47.9	62.1	35.2	14.2
April 6-11	48.7	63.2	38.6	15.5
April 13-20	50.7	63.7	32.1	12.5
April 20-25	52.5	64.8	32.5	14.9
April 27-May 2	54.3	66.7	34.4	18.0
May 4-9	56.5	70.9	39.3	17.7
May 11-16	57.0	72.3	35.1	22.5
May 18-23	60.0	75.0	29.1	19.0
May 25-30	63.6	79.8	19.4	21.8
June 1-6	65.6	82.2	23.3	16.3
June 8-13	65.9	84.5	16.8	15.7
June 15-20	67.6	86.5	12.3	14.3
June 22-27	69.3	88.6	9.1	14.4
June 29-July 4	70.3	88.1	1.2	11.9
July 6-11	71.0	89.3	8.3	17.0
July 13-17	73.3	92.0	11.2	12.3
July 20-24	73.6	92.2	0.0	10.8
July 27-29	73.8	86.9	0.0	10.8

The conclusions of Reed and Faris regarding the effect of environment on sorghum smut infection obtained under controlled conditions in the greenhouse are substantiated by the studies made under field conditions at Manhattan, Kansas.

Soil temperature and soil moisture are interdependent factors in determining infection.

Medium to low soil temperatures with medium to low soil moistures seem to be most conducive to the maximum infection.

Fair infection occurs over a very wide range of soil temperatures and soil moistures. Heavy sorghum kernel smut infection occurs at any temperature below 75°F. which allows the seed of kafir to germinate; in fact, low temperatures consistently are associated with high smut infection. An absolute minimum temperature of 28°F. during the infection period is no hindrance to infection.

It appears that low soil moisture (i.e., below 8 or 10 per cent on the dry basis) reduces the amount of infection; however, when the soil moisture remains at this point, sorghum seed will not germinate. When the soil moisture becomes sufficient for seed germination, following rains, then some infection may occur. This indicates that in as far as soil moisture is concerned, smut infection may occur whenever the seed of sorghum germinates readily.

It is interesting to note that kafir may be planted earlier than has been generally thought possible without losing the entire stand from freezing. In the five years' work, the early plantings in March were not killed by freezing.

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MITOCHONDRIA AND PLASTIDS IN LIVING CELLS OF ALLIUM CEPA¹

Helen Sorokin

THIS INVESTIGATION presents evidence which demonstrates that it is possible to differentiate mitochondria from morphologically similar forms of plastids. In addition, it records the results of a study not only of the vital staining of mitochondria but also of the effects of various liquid media upon the survival of living tissue cells and upon the form and behavior of mitochondria and of plastids.

MATERIAL AND METHODS.—The epidermal tissue of the adult scale leaves of *Allium Cepa* L. provides unusually favorable material for microscopic examination and may be removed without seriously injuring the living cells. Small sheets of the epidermis were peeled from the inner surfaces of the scale leaves and were floated, with the cuticular side uppermost, upon various liquids. The behavior and the survival of the living cells were tested upon tap water, distilled water, mineral oil, solutions of sucrose varying from 2.5 to 30 per cent, and a series of Clark's standard M/20 buffers.² The liquids upon which the tissue cells survived the longest were subsequently utilized in vital staining with Janus green B. A pre-war sample of Hoechst's dye was used in a concentration of one drop of a 1 per cent aqueous solution of the dye to 50 cc. of the liquid medium. With the exception of the stock solution of the Janus green B, all other solutions were freshly prepared at the beginning of each experiment. The cytological criteria outlined by Bailey and Zirkle (1931) were employed

in determining the condition of living tissue. All critical examinations were made upon cells which exhibited normal cyclosis.

SURVIVAL OF TISSUE CELLS.—During the months of November, December, January, and February, tap water from certain specific localities provides one of the most favorable media for the survival of tissue cells. Bailey and Zirkle (1931) found that cambial initials exhibit normal cyclosis for from 1200-1700 hours when immersed in tap water from Forest Hills, Boston, but subsequently discovered that such was not the case in dealing with tap water from other sources—e.g., Cambridge, Mass. My experience, in dealing with the epidermal tissue of onion, has been that tap water from Forest Hills, Boston, and from Winchester, Mass., is particularly favorable; whereas that from Cambridge, Mass., is quite unsatisfactory. Epidermal cells survive for a period of approximately 18 days in Winchester tap water.³

Few epidermal cells survive for more than 12 days in distilled water. In mineral oil and in solutions of sucrose varying in concentration from 2.5 to 30 per cent, the cells exhibit active protoplasmic streaming after 150 hours and may at times survive for 14 days or more. The lethal effects of Clark's standard M/20 buffers are obvious, and the epidermal cells of the onion seldom remain alive for any considerable length of time. The longest survival was 48 hours in the pH 7.8 and pH 8.2 borates. In the pH 5.4 phthalate, the cells survived for 30 hours; in the pH 5.8 phthalate, for 24 hours; in the pH 5.8 phosphate, for 26 hours; and in the pH 6.2 phosphate, for 20 hours. The more acid phthalates and the more alkaline phosphates and borates are extremely toxic.

³ The chemical analysis of this water for January 1937, as given by the State Department of Health, is as follows: chlorides, 4.4; nitrates, 0.05; iron, 0.15; in parts per million. Hardness, 20. The pH at 6°C. was 7.8.

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² The following series of buffers were used: pH 3.0, 4.0, 4.6, 5.0, 5.4, 5.8 (phthalates); pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8 (phosphates); pH 7.8, 8.2, 8.6, 9.0 (borates).

VITAL STAINING WITH JANUS GREEN B.—Laguesse (1912) observed in the living cells of various vertebrates and invertebrates an elective affinity of mitochondria toward Janus green B, but indicated that the staining is erratic. E. V. Cowdry (1914, 1916) emphasized that Hoechst's Janus green B—i.e., diethylsafraninazodimethylanilin chloride—stains mitochondria specifically and that the contradictory results obtained with other samples of Janus green may be attributed to admixtures of other dyes. The specificity of Hoechst's dye depends upon the diethylsafranin group. The differential staining of animal mitochondria by Hoechst's Janus green B has been confirmed by a number of zoologists, and the use of this dye has become a standard procedure in intravital staining (Ludford, 1933).

N. H. Cowdry (1917) is credited with having first successfully applied this test to plant mitochondria. He found that they react to it precisely as do animal mitochondria except that the staining is much slower, owing to difficulties in penetration of the dye into plant cells. According to Guilliermond (1923), plant mitochondria stain with Janus green B while the cells are alive, but he concluded that the mitochondria undergo rapid degeneration shortly after staining and that the dye has pronounced lethal effects upon the cells. In the mycelium of *Endomyces magnesi* only, the mitochondria remained intact for several hours after staining. In accordance with his theory that the "active" and "ordinary" chondriosomes have identical physical and chemical properties, Guilliermond emphasized that leucoplasts also stain with Janus green B, but that they do so more slowly.

Opinions vary concerning the extent to which Janus green B is injurious to living cells. E. V. Cowdry (1914) observed stained mitochondria in neurophile leucocytes during amoeboid movement and phagocytosis. Becker (1933) described the formation of the phragmoplast and the phenomena of cytokinesis in cells of *Tradescantia* that were stained with Janus green B. Lewis and Lewis (1924) concluded that the dye produces conspicuous abnormalities in animal cells after 15 minutes. Parat (1928), although considering Janus green B as one of the best reagents for the staining of mitochondria, considered the staining reaction to be post-vital.

If the epidermal cells of the onion are immersed in tap water containing Janus green B in a ratio of approximately 1 to 100,000 or in a 12 per cent aqueous solution of sucrose containing a similar ratio of the dye, the staining of mitochondria is extremely erratic. If sheets of epidermal tissue are floated with the cuticular surface down upon such solutions of the dye, the tissue becomes colored along the margins only. The cuticle prevents the dye from penetrating into the cells, and the mitochondria remain unstained except in cells at the periphery of the piece. On the contrary, if the epidermis is floated with the cuticular surface uppermost, the mitochondria of the living cells stain after 45–60 minutes and remain colored as long as the cells survive—i.e., for about 30

hours. It is essential, however, to transfer the sheets of tissue from time to time to a freshly prepared solution, since the dye is likely to precipitate upon standing, and the solutions become colorless. After 12 hours, or sometimes even sooner, the contents of the vacuoles and the cell walls take up the dye and tend to obscure the staining of the mitochondria.

The mitochondria may be uniformly colored a bright blue-green (fig. 1, 4, 6), or they may exhibit deeply stained granules in an uncolored matrix (fig. 2). The latter type of staining occurs commonly in cells which do not survive so long as those in which the mitochondria are uniformly colored. A study of living and of fixed material indicates that mitochondria normally are microscopically homogeneous structures and that a differential staining of granules and matrix is due to an abnormal segregation of two components of the mitochondria.

When living epidermal cells, containing colored mitochondria, are mounted under a cover glass in a drop of dye solution and are examined under an oil-immersion lens, the color suddenly disappears after a varying interval of time from all the mitochondria, and the cells assume the appearance of a freshly prepared, unstained mount. If the destained cells are floated again upon the dye solution, the mitochondria restain, and the process of staining and destaining may be repeated a number of times. This capacity for staining and destaining is characteristic of normal uninjured mitochondria and may be utilized in distinguishing mitochondria from other stainable bodies, such as the granular precipitates that occur at times within or upon the surface of vacuoles. The behavior of the epidermal cells of onion suggests that the staining and destaining of mitochondria may be correlated with variations in the available supply of oxygen.

The staining of mitochondria with Janus green B in distilled water is erratic and uncertain, and it varies markedly in different buffers of Clark's standard series. There is no staining in buffers more acid than pH 4.6 or more alkaline than pH 8.2. In the case of the phthalate series, the mitochondria stain well for 30 hours at pH 5.4 and for a somewhat shorter period of time at pH 5.8. In the phosphate series, the mitochondria exhibit an excellent coloration for 26 hours at pH 5.8 (fig. 6) and for decreasing periods of time at pH 6.2, pH 6.6, and pH 7.0. There is little, if any, staining at pH 7.4 and pH 7.8. In the borate series, the mitochondria stain well at pH 7.8 and fairly well at pH 8.2. It is evident that mitochondria are capable of staining with Janus green B over a comparatively wide range of hydrogen-ion concentrations and that their staining behavior is largely dependent upon the toxicity of specific buffers.

In none of the living epidermal cells that I have examined have I encountered evidence of the vital staining of plastids—regardless of variations in their size and form—with Janus green B (fig. 1, 3, 5, 6). Staining of the plastids occurs in obviously dying or dead cells only. A truly vital staining with Janus green B, therefore, may be utilized as a means of dif-

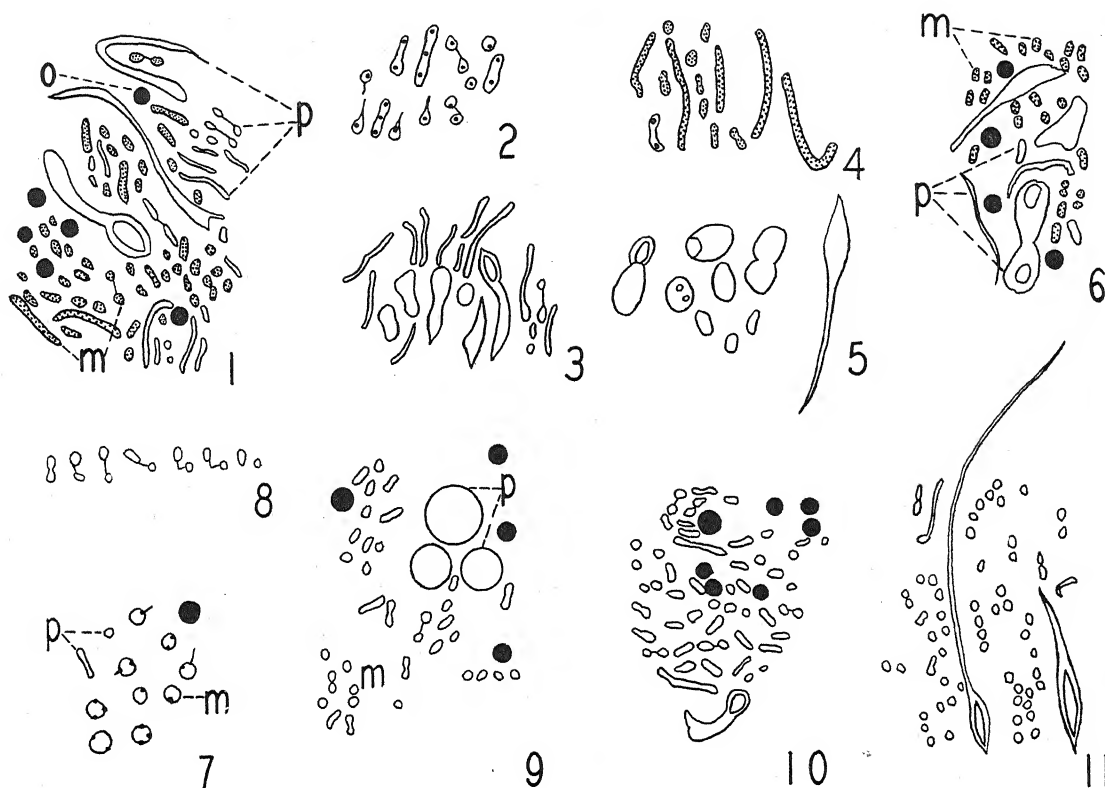


Fig. 1-11. Mitochondria and plastids from living cells. m=mitochondria, p=plastids, o=oil globules. Magnification approximately 2000.—Fig. 1. Stained for 50 minutes with Janus green B in a 12 per cent solution of sucrose in tap water: mitochondria stained, plastids colorless.—Fig. 2. Stained as above for 5 hours; mitochondria with deeply stained granules in a colorless matrix.—Fig. 3. Same treatment as in fig. 2; unstained plastids.—Fig. 4. Stained as in fig. 1 for 12 hours; many of the mitochondria are very long and uniformly stained.—Fig. 5. Treatment as in fig. 4; plastids unstained.—Fig. 6. Stained for 24 hours with Janus green B in pH 5.8 phosphate buffer; mitochondria stained, plastids colorless.—Fig. 7. Stained for 30 minutes with Janus green B in a 7.5 per cent solution of sucrose in distilled water; vesiculation of mitochondria which exhibit deeply stained granules, plastids unstained.—Fig. 8. Successive stages in the division of a mitochondrion.—Fig. 9. From a cell kept in a 30 per cent, hypertonic solution of sucrose for 6 days; mitochondria of normal form, plastids spherical.—Fig. 10. Mitochondria and plastids from a cell kept in isotonic solution for 10 days.—Fig. 11. From a cell kept in tap water for 13 days; mitochondria predominantly of granular form, starch-containing plastids with slender elongated processes.

ferentiating mitochondria from morphologically similar forms of plastids. It should be emphasized in this connection, however, that when living epidermal cells of onion are floated upon sugar solutions, the larger plastids frequently become transformed into chloroplasts and thus exhibit a green coloration.

TERMINOLOGY.—Lack of uniformity in the terminology used by different cytologists in designating specific components of the cytoplasm leads to considerable confusion in the literature. A number of botanists employ the terms *chondriome*, *chondriosome*, and *chondriocont* in referring to both mitochondria and plastids. Zirkle (1929) and Anderson (1936) include under mitochondria all small cytoplasmic inclusions that are preserved by bichromates on the basic side of pH 4.2-5.2 and are destroyed by more acid fixatives. This definition of the term includes both mitochondria and the "primordia of plastids." It seems advisable to use the term *mitochondria* to designate granular and short or long rod-shaped components

of the cytoplasm which stain in the living condition with a dilute solution of Janus green B. According to this usage, the term *mitochondria* does not include the "primordia of plastids," since these bodies do not stain with Janus green B in normal, uninjured living cells. In so far as the Janus green B test is concerned, the mitochondria of plant cells may be homologized with the mitochondria of animal cells, as defined by E. V. Cowdry (1916) and others.

FORM AND BEHAVIOR OF MITOCHONDRIA AND PLASTIDS.—Although their index of refraction is rather low, mitochondria are clearly visible in living epidermal cells of the onion without vital staining. They occur in the form of slightly asymmetrical granules, diplosomes, short rods (length of 2 to 3 times the diameter) (fig. 1, 10), and long rods (length up to 15 times the diameter) (fig. 4). The small granular forms are fairly uniform in size and never extend down to the limits of microscopic visibility. The outline of the rod-shaped forms is smooth, the diam-

eter is relatively constant, and the ends are blunt except in the case of rods which have just passed through a diplosome stage of transverse division. Conspicuous changes in the girth and form of mitochondria, such as are recorded by Emberger (1927) and others, do not occur in normal living cells of the onion but are characteristic of plastids. Under normal conditions the mitochondria were neither observed to shorten and to become thicker nor to elongate and become thinner. There was no evidence of branching of mitochondria in the cells of onion nor of the formation of networks.

Diplosomes commonly represent stages in the division of mitochondria and were present in all the living cells examined. The actual process of division was frequently observed both in freshly prepared mounts and in material kept *in vitro*. During division (fig. 8), the threadlike connection between the halves of the diplosome is stretched and suddenly ruptured. The halves of the diplosome jerk apart as if the connecting thread broke under considerable tension. The interval of time that elapses between the formation of a diplosome and the completion of a division is extremely variable. It is possible that certain of the diplosomes may, at times, reassume a rod-shaped form without undergoing division. When the length of a rod is more than twice its diameter, the division may not be equatorial. Thus, I have observed divisions in which a spherical body is cut off from one end of a long rod-shaped mitochondrion.

It should be noted in this connection that, although various investigators have described and figured diplosomes, or so-called dumb-bells, as stages in the division of mitochondria, relatively few observations of the actual process of division in normal living cells are recorded in the botanical literature. Friedrichs (1922) worked to some extent with living material of *Elodea*, but it is not clear from his descriptions whether he observed actual divisions or based his conclusions merely upon the occurrence of supposedly transitional stages. Kassman (1926) gives an accurate description of the division of small granules, presumably mitochondria. However, certain of these granules gave a positive test for starch, which indicates that Kassman may have been concerned with small plastids rather than with mitochondria. Horning (1926) followed the process of binary fission in living mitochondria of a heretotrichan infusorian, vitally stained with a sodium salt of diethylsafranin monocarboxylic acid. His descriptions of the process of division, as also Guilliermond's (1927) based upon plant material, are in close agreement with the phenomena that I have observed in living epidermal cells of the onion.

The plastids of the epidermal tissue occur in a wide variety of forms (fig. 3), but they never grade down to the limits of microscopic visibility. The small granular forms are slightly smaller than the granular forms of mitochondria, and the short rod-shaped plastids are more slender than the mitochondria of corresponding length. There are "dumb-bell"-shaped

plastids which resemble diplosomes. These "hantelförmige Gebilde" are easily confused with the diplosome forms of mitochondria and have served as one of the principal arguments for deriving plastids from mitochondria (Cunha, 1929; Loui, 1930). There are, in addition, numerous transitional forms between small and large plastids. The smaller plastids may be observed to develop into large ones, and conversely the latter may at times divide to form small plastids (compare Guilliermond et al., 1933, p. 147).

The behavior of the granular and short rod-shaped forms of mitochondria and of plastids in living epidermal cells which exhibit normal cyclosis is similar, except for the fact that mitochondria stain vitally with Janus green B, whereas the plastids do not. The larger forms of plastids appear to be less viscous and exhibit amoeboid movements and striking changes of form when kept under observation for a considerable interval of time; whereas the longer rod-shaped mitochondria are pliable but of relatively constant diameter throughout their length.

EFFECTS OF HYPOTONIC AND HYPERTONIC SOLUTIONS.—Fauré-Fremiet (1910), Lewis and Lewis (1915), Bang and Sjövall (1916), N. H. Cowdry (1917), Guilliermond (1919), Anitschkow (1923), and Kamenev (1934) are of the opinion that mitochondria swell and become vesiculate in hypotonic solutions and that they shrink and become slender in hypertonic media. Unfortunately, it is not possible to determine whether the changes observed by these investigators are due directly to osmotic phenomena or to the effects of a complex of factors in injured or dying cells. In a heterogeneous system, such as a living cell, it is extremely difficult to distinguish the effects of various factors influencing the form and the behavior of mitochondria, and specific changes may be due to injury rather than to the direct effects of variations in osmotic pressure. It is significant in this connection that Bang and Sjövall record cases of the swelling and vesiculation of mitochondria in isotonic solutions and note that such changes are concomitants of the degeneration of mitochondria.

The mitochondria of uninjured epidermal cells of onion do not swell or become vesiculate in tap water (fig. 11), distilled water, or in dilute solutions of sucrose, nor do they exhibit conspicuous contraction in saccharine solutions of 15–30 per cent. On the contrary, the mitochondria of injured or dying cells frequently swell and vesiculate when the cells are placed in hypotonic solutions, but they may do so at times in isotonic (fig. 7) or hypertonic solutions. The specific degenerative changes that the mitochondria undergo are extremely variable and are apparently dependent upon the condition of the "explanted" cells at the time when they are transferred to specific solutions.

Division or fusion of mitochondria may be induced at times in living cells that are transferred to culture solutions. I have observed these phenomena particularly in the case of distilled water. In certain cases all the rod-shaped mitochondria are resolved by divi-

sion into spherical forms within ten minutes after transfer to distilled water. Subsequently, the granular forms begin to coalesce and after 20 minutes may fuse and restore the original rod-shaped forms. When the distilled water contains Janus green B, the elongated mitochondria frequently divide, but I have not observed a subsequent fusion of spherical forms.

In contrast to the mitochondria, the plastids of uninjured cells tend to assume more nearly spherical forms in hypertonic solutions (fig. 9) and slender, threadlike or branching forms in hypotonic ones (fig. 11). Such changes of form are not due solely to division or fusion of plastids, but primarily to variations in the shape of individual leucoplasts, which may involve conspicuous expansions and contractions.

DISCUSSION.—There exists much diversity of opinion regarding the origin of mitochondria and plastids (see Guilliermond, 1927; Sharp, 1934). Those who maintain that both categories of bodies originate *de novo* have argued that (1) both mitochondria and plastids grade down in size to the limits of microscopic visibility and (2) both of them may be observed at times to appear and to disappear from the cytoplasm. The former argument appears to be based upon a failure to differentiate mitochondria and plastids from various minute granules of ergastic substances. In none of the living cells of varied monocotyledons and dicotyledons that I have examined do the mitochondria or plastids grade down in size to the limits of microscopic visibility. Furthermore, as Kassman (1926), Guilliermond (1927), Faull (1935), and others have observed, the cytoplasm of living plant cells, when examined under the microscope, frequently exhibits successive phases of clarity and opacity. During the periods of opacity, the mitochondria, and even relatively large plastids, may be obscured and thus become invisible. In other words, the putative appearance and disappearance of mitochondria and plastids may be due entirely to changes in visibility and can not be interpreted as a convincing demonstration of the *de novo* origin of these bodies.

The available evidence indicates that both mitochondria and plastids divide and, therefore, are capable of self-perpetuation. In the case of the epidermal tissue of the onion, it is evident that the two categories of bodies, although morphologically similar, exhibit detectable differences in size and form and may be distinguished in uninjured living cells by their differential staining in Janus green B. It is essential to determine whether they exhibit a similar behavior in the case of other tissues and of other plants. In

order to test this point, I have studied the living cells of a considerable number of monocotyledonous and dicotyledonous plants. In certain of them the granular and rod-shaped plastids contain starch, oil, or even pigments and therefore cannot be interpreted merely as non-vital staining forms of mitochondria. In others the plastids exhibit such marked and constant differences in size and form that they cannot be confused with mitochondria. As will be shown in a subsequent paper, the mitochondria stain vitally with Janus green B, whereas plastids do not. The living cells of different tissues and plants vary greatly, however, in their sensitivity to injury and in their reactions during specific experimental treatments. The exact procedure in the vital staining of mitochondria must, therefore, be varied in dealing with different types of material.

SUMMARY

The survival of living epidermal cells of onion was tested in tap water, distilled water, mineral oil, saccharine solutions of varying concentrations, and a series of Clark's standard buffers, both with and without Janus green B.

The mitochondria of uninjured living cells stain vitally with Janus green B, whereas plastids of similar morphological forms do not. However, in order to demonstrate a truly vital staining of mitochondria, the experimental procedure must be carefully controlled. Not only is it essential to utilize the right type of dye in proper dilution, but also to provide an adequate supply of oxygen and conditions favorable for the penetration of the dye and for its accumulation within the mitochondria.

In the case of uninjured living cells, the mitochondria do not swell and vesiculate in hypotonic solutions nor contract conspicuously in hypertonic ones. On the contrary, the plastids frequently modify their form and surface area when the cells are transferred to hypotonic and hypertonic solutions.

The available evidence indicates that there are two categories of self-perpetuating bodies—i.e., mitochondria and plastids, which may be differentiated in uninjured living cells by their reactions towards Janus green B. Neither mitochondria nor plastids grade down in size to the limits of microscopic visibility. The putative appearance and disappearance of these bodies when living cells are examined under the microscope are due to successive phases of clarity and opacity of the cytoplasm.

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CONDITIONING SEEDS TO TOLERATE SUBMERGENCE IN WATER¹

H. Clyde Eyster

THE REACTION of seeds to soaking during submergence in water has been studied by several investigators. The most extensive and best known publications on this subject are those of Kidd and West (1918-1919, 1919) and of Bailey (1933).

Kidd and West soaked seeds of culinary pea, dwarf bean, broad bean (*Vicia Faba*), sunflower, wheat, oats, white mustard, and white lupine under 4 cm. of distilled water at an average temperature of 17°C. in the laboratory for periods varying from 6 to 72 hours. They concluded that seeds soaked in distilled water, previous to sowing, frequently developed into plants with decreased growth and yield, that a germination test could not be relied upon in the least to give any criterion of what the decrease in growth and yield might be, and that the nature of the effect

was strongly specific. It was shown in this regard that quite different results were obtained with similar treatments upon the seeds of closely allied plants.

Bailey (1933) soaked seeds of Early Valentine bean in water which was both sterile and aerated. Each day 24 liters of air were drawn through a flask containing the sterile water and seeds. The seeds were sterilized in the same flask by placing them in a 0.25 per cent Uspulun solution for fifteen minutes. The sterilizing solution was decanted, and the flask and seeds were then washed thoroughly with sterile distilled water. Bailey found that soaked seeds showed a decrease in germination, that there was a further decrease in the number of mature plants which developed, and that the soaking treatment resulted in a progressive decrease in the growth and weight of the plants produced, increased the time required to reach maturity, and produced a modification in the structure of the leaf. Since there was no decrease in the quantity of carbon dioxide respired, he concluded that the soaking treatment did not injure the

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respiratory enzymes and that the reduction in growth was not due to a decrease in the rate of respiration.

Coupin (1898) has shown that some seeds can withstand only a short period of immersion in water and that others can tolerate a long one.

Tilford, Able, and Hibbard (1924), also, have studied the reaction of seeds to soaking, but their experiments deal primarily with the cause of decreased germination of seeds which have been soaked in water. They soaked seeds of the common navy bean first in stagnant and in running water and then in water under absolutely sterile conditions. Seeds which had been soaked in running water showed better germination than those which had been soaked in stagnant water, and seeds which had been soaked in water under sterile conditions produced a higher percentage of germination than seeds which had been soaked similarly in water under aseptic conditions. They concluded that bacterial activity was the main factor in the devitalization of the bean seeds.

Direct studies on the problem of conditioning seeds to tolerate submergence in water have probably never been reported.

MATERIALS AND METHODS.—Brittle wax bush bean seeds, kidney wax bush bean seeds, and stringless green pod bush bean seeds were the principal kinds of seeds used. In a few experiments lima bean seeds, Canada field pea seeds, Gradus pea seeds, and soybean seeds were also used. All the seeds except the soybeans were purchased from W. Atlee Burpee Seed Co.

Seeds were conditioned to tolerate submergence in water by placing the seeds on moist porous blocks of fired clay at room temperature for two days. Deviations from this method of conditioning seeds to tolerate submergence in water were also used. These deviations will be described later. Seeds subjected to a conditioning process will be referred to in this paper as "conditioned" seeds.

Seeds to be soaked were placed in ordinary glass tumblers in distilled water which was kept at a constant temperature. For the regulation of temperature, cases which varied no more than 1°C. on either side of the recorded temperature were used. Twenty seeds were placed in 100 ml. of distilled water. In order to have a way to check the data, two sets of twenty seeds each were treated separately, similarly, and simultaneously in as many cases as convenient.

After the soaking process the seeds were germinated, and the seedlings were grown for a short time on moist porous blocks of fired clay. In a few cases seeds were planted in the soil in the greenhouse bench at a uniform distance apart and at a uniform depth of one inch.

DATA AND RESULTS.—Kidney wax bush bean seeds were conditioned to tolerate submergence in water at 10°C. by first placing them on moist porous blocks of fired clay at room temperature for two days. These seeds swelled, due to the imbibition of water from the moist porous clay blocks, and increased in enzymatic and respiratory activity. Table 1 gives the

TABLE 1. Germination data for kidney wax bush bean seeds (1935 crop) which has been previously soaked in water at 10°C.

Condition of seeds before soaking	Length of soaking period, days	Germination (out of 20)
"Conditioned"	3	19
"Conditioned"	3	19
"Conditioned"	4	16
"Conditioned"	4	20
"Conditioned"	5	19
"Conditioned"	5	20
"Conditioned"	10	16
"Conditioned"	10	16
"Conditioned"	15	13
"Conditioned"	15	14
"Unconditioned"	3	6
"Unconditioned"	3	5
"Unconditioned"	4	2
"Unconditioned"	4	6
"Unconditioned"	5	2
"Unconditioned"	5	4
"Unconditioned"	10	0
"Unconditioned"	10	0
"Unconditioned"	15	0
"Unconditioned"	15	0

results of an experiment in which both "conditioned" and "unconditioned" bean seeds were soaked in water at 10°C. for periods of time varying from three days to fifteen days. The germination of "conditioned" seeds after being soaked at 10°C. for three, four, and five days, respectively, was almost 100 per cent, and the seedlings were exceptionally vigorous. A very small number of "unconditioned" bean seeds germinated after being soaked in water at 10°C. for the same periods of time. "Conditioned" seeds soaked for 10 and 15 days, respectively, showed decreases in the number of seeds which germinated. The decreases, however, were very small, as can be noted in table 1. None of the "unconditioned" seeds germinated after being soaked in water at 10°C. for ten days or longer.

Stringless green pod bush bean seeds of the 1934 crop could be conditioned to tolerate submergence in water at 10°C. by first placing them on moist porous clay blocks at room temperature for only one day. These seeds were very permeable and imbibed water rather rapidly from the moist porous clay blocks.

Seeds of lima bean, of Manchu soybean, of Gradus pea, and of Alaska pea, also, could be conditioned to tolerate submergence in water at 10°C. by first placing the seeds on moist porous blocks of fired clay at room temperature for two days. The germination results, which are recorded in table 2, are fundamentally similar to those for the kidney wax bush bean seeds. The seeds of lima bean are very sensitive to the presence of excessive moisture. It can be observed in table 2 that "unconditioned" lima bean seeds did not tolerate submergence in water at 10°C. for three days, while "conditioned" lima bean seeds

soaked under similar conditions for the same length of time gave 100 per cent germination. "Unconditioned" seeds of the Alaska pea were fairly resistant to submergence in water at 10°C., and it was only after four days' soaking at 10°C. that a decrease in germination occurred. Seedlings which were grown from seeds soaked in water at 10°C. were always more vigorous whenever the seeds were previously conditioned to tolerate submergence in water.

The most satisfactory and the most extensively used method of conditioning seeds to tolerate submergence in water at 10°C. has already been described. It was accomplished by placing the seeds

weeks at 10°C. During the conditioning process the seeds imbibed water from the moist porous clay blocks and increased in size but failed to germinate at 10°C. Brittle wax bush bean seeds (1935 crop) conditioned by this last mentioned method and then submerged in water at 10°C. for five days germinated remarkably well. For two trials of twenty seeds each the germination was 16 and 19, respectively.

Bean seeds conditioned to tolerate submergence in water at 10°C. were found by experiment to be proportionally less resistant to submergence in water at higher temperatures. It is probably because respiration is at a low ebb at 10°C. that "conditioned"

TABLE 2. Germination data for various species of seeds (1935 crop) which had been soaked in water at 10° C.

Species of seeds	Condition of seeds before soaking	Length of soaking period, days	Germination (out of 20)
Lima bean	"Unconditioned"	3	1
Lima bean	"Conditioned"	3	20
Manchu soybean	"Unconditioned"	3	9 ^a
Manchu soybean	"Unconditioned"	3	6 ^a
Manchu soybean	"Conditioned"	3	20
Manchu soybean	"Conditioned"	3	20
Gradus pea	"Unconditioned"	3	4 ^b
Gradus pea	"Unconditioned"	3	8 ^b
Gradus pea	"Conditioned"	3	18
Gradus pea	"Conditioned"	3	12
Alaska pea	"Unconditioned"	3	20
Alaska pea	"Unconditioned"	3	19
Alaska pea	"Conditioned"	3	20
Alaska pea	"Conditioned"	3	20
Alaska pea	"Unconditioned"	4	14 ^b
Alaska pea	"Unconditioned"	4	15 ^b
Alaska pea	"Conditioned"	4	20
Alaska pea	"Conditioned"	4	19

^a Very weak seedlings. ^b Moldy seedlings.

on moist porous blocks of fired clay for two days at room temperature. Two other methods of conditioning seeds were found to be successful. First, seeds were conditioned to tolerate submergence in water at 10°C. by previously submerging twenty seeds in 100 ml. of water at room temperature until they were swollen. Stringless green pod bush bean seeds (1934 crop) were soaked for six hours in water at room temperature and then submerged in water at 10°C. for two days. The majority of "conditioned" seeds always germinated, whereas only a small proportion of the "unconditioned" seeds germinated. The data for a specific experiment show that for the "conditioned" seeds submerged in water at 10°C. for two days 15 out of 20 and 18 out of 20 seeds, respectively, germinated. "Unconditioned" seeds soaked similarly and simultaneously for the same length of time gave a germination of 6 out of 20 for each of two trials. Secondly, seeds were conditioned to tolerate submergence in water at 10°C. by previously placing the seeds on moist porous blocks of fired clay for two

seeds could tolerate submergence in water at 10°C. As the temperature of the water in which the seeds were submerged was raised, respiration increased rapidly and the "conditioned" seeds became less tolerant of the soaking process.

An experiment was performed to determine the rate of water absorption and the total amount of water absorbed by "conditioned" seeds. The data show that the rate of water absorption by "conditioned" seeds during submergence in water at 10°C. is as fast or even faster than that by "unconditioned" seeds during similar conditions. The total amount of water absorbed was in most cases slightly greater for the "conditioned" seeds than that for the "unconditioned" seeds.

By additional experimentation it was discovered that seedlings with radicles $\frac{1}{4}$ inch to 1 inch in length could not tolerate submergence in water at 10°C. as well as the "conditioned" seeds could. Seedlings submerged in water at 10°C. for one day lost the tip of the primary root. Seedlings submerged for more

than one day were proportionally more and more stunted in growth. The root from the tip to the region of cell differentiation became soft and died, and numerous branch roots soon appeared and continued to grow.

A brief study was made to determine the influence of dilute solutions of various salts on the germination of "conditioned" seeds which had been previously submerged in water at 10°C. Some of the salts were disinfectants while others were ingredients of fertilizers. The particular chemicals used and their concentrations were as follows: sodium sulphate, 1.0 per cent of saturation; monobasic calcium acid phosphate, 1.0 per cent of saturation; copper sulphate, .01 per cent; chloramine T, .01 per cent; potassium nitrate, .01 per cent; and potassium chloride, .01 per cent. It was found that "conditioned" seeds could tolerate submergence in these aqueous salt solutions fairly well.

PRACTICAL APPLICATIONS.—Frequently seeds are submerged in aqueous solutions of disinfectants in order to sterilize the seed coats. Lengthy submergence of "unconditioned" seeds in dilute aqueous solutions of disinfectants is not recommended. Applications of disinfectants in the form of dry dust is a much better method of sterilizing seeds. Whenever submergence in aqueous solutions is absolutely necessary, the seeds should first be conditioned to tolerate such submergence.

Seeds are occasionally soaked in water to hasten germination. This practice is not advisable whenever dormancy of the seeds is not due mainly to an impermeable seed coat. Soaking tends to decrease the vigor and yield as well as the percentage of germination. To hasten germination, it is recommended that the seeds be placed on cloth or paper towels continually moistened for one or two days at room temperature and that they then be planted in the soil before the radicle protrudes.

Soybeans sown in the spring immediately before a cold rain frequently show marked reductions in germination and in vigor and yield. Remarkable differences are often to be observed between portions of the field sown before the cold rain and portions sown several days later. The recommendation to the farmer and to the gardener is that they do not sow seeds in the spring whenever indications are that there will be a cold rain within the next 24 hours. In some cases it will be convenient and advisable for the gardener to condition the seeds previous to sowing in order to insure them against decay and against a reduction in the vigor and yield of the plants. This conditioning is accomplished by placing the seeds on continually moistened cloth or paper towels at ordi-

nary room temperatures for one or two days, and then by planting them in the soil before the radicle protrudes.

The ecologist, also, may find that the principle emphasized in this paper explains the distribution of numerous species of plants.

SUMMARY

Bean seeds were conditioned to tolerate submergence in water at 10°C. by previously placing the seeds on moist porous blocks of fired clay for 2 days at room temperature. "Conditioned" kidney wax bush bean seeds could tolerate 15 days of submergence in water at 10°C. without much reduction in germination. "Unconditioned" kidney wax bush bean seeds could not tolerate more than about 5 days of submergence in water at 10°C.

"Conditioned" and "unconditioned" seeds of lima bean, Manchu soybean, Gradus pea, and Alaska pea gave results basically similar to those obtained for the kidney wax bush bean seeds.

The rate of water absorption by "conditioned" seeds is as fast or even faster than that by "unconditioned" seeds. The total amount of water absorbed is in most cases slightly less for the "unconditioned" seeds.

Seedlings with radicles $\frac{1}{4}$ inch to 1 inch in length were stunted by submergence in water at 10°C.

"Conditioned" seeds were found, also, to tolerate submergence in dilute aqueous solutions of salts at 10°C.

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NORMAL GROWTH, REGENERATION, AND ADVENTITIOUS OUTGROWTH FORMATION IN FERN PROTHALLIA¹

Harry G. Albaum

THE SYMMETRICAL heart-shaped fern prothallium provides an excellent subject for studies on growth. It has a simple form and a relatively small number of cells; it thrives under conditions which are easy to maintain; it can be cut very easily in order to study growth changes.

Since the middle of the last century an extensive literature dealing with the growth and development of the fern prothallium has been published (see Dopp, 1927; Orth, 1936). Few of these accounts, however, present material which can be used in making quantitative analyses of the growth process. Most of the previous work has been concerned with a purely descriptive analysis of the early growth of various prothallia.

Dopp (1927) traced the "cell lineage" of a single prothallium of *Onoclea struthopterus* which he was able to follow over a period of 47 days. From his data significant quantitative relations of the normal growth process may be worked out.

Of further interest is the phenomenon described by Heim (1896), Goebel (1905), and Linsbauer (1926). When cells of the prothallium which come from the apical region or close to it are isolated by cutting, they continue to grow. Here again no data are available which permit a correlation of this type of growth with the growth of the whole prothallium from the point of view of mass and form relations.

Prothallia which lack an apical region develop adventitious sprouts (Heim, 1896; Goebel, 1905; Linsbauer, 1926). These are derivatives of single inner or marginal cells which grow out in a manner analogous to the outgrowth of a prothallial filament from its spore. In many cases such sprouts produce an apical region and eventually may give rise to a complete prothallium with sex organs capable of functioning in fertilization. Adventitious sprouts rarely appear on young, actively growing, uninjured prothallia under optimal conditions of light, temperature, and humidity. They were first observed by Hofmeister (1851) on abortive prothallia of *Notochlaena*, *Allosorus*, and *Gymnogramme* where they arose from border cells. Since then they have been seen in many fern genera. They have appeared on prothallia subjected to a variety of abnormal conditions: (1) grown close together; (2) isolated from old cultures; (3) placed under lights of different wave length; (4)

treated with moderate dosages of X-rays; (5) grown under the surface of water; (6) placed for a time in chambers containing an atmosphere of ether and chloroform; (7) plasmolyzed and then deplasmolyzed; (8) injured accidentally or by cutting. No quantitative data on the growth of adventitious prothallia are to be found in the literature.

The experiments to be described were undertaken with the following objectives: (1) to study the quantitative relations in normal growth with respect to form change, cell size and number, localization of growth, relationship of mass to increase in size, and form change after reorientation with respect to light; (2) to study quantitatively the behavior of apical regions when isolated from the rest of the prothallium, and the behavior of adventitious sprouts which arise from basal pieces removed from intact prothallia by cutting.

MATERIAL AND METHODS.—Two species of *Pteris* were used in these investigations, *Pteris aquilina* and *Pteris longifolia*. The spores of the former were collected in the vicinity of the Marine Biological Laboratories at Woods Hole, Mass. The spores of the latter were secured through the courtesy of Dr. Ralph C. Benedict of the Brooklyn Botanic Garden.

The germination of the spores was carried out in the following way: Clean 4-inch flower pots were filled with sterile sphagnum moss. These were then inverted and placed in large dishes in which the water level was kept constant. The spores were sprinkled on the surface of the inverted pots, and the dishes containing them were covered. The latter were placed where they would receive ample light (either upon a window sill or in the greenhouse). Germination in all cases occurred within a week.

Prothallia were isolated from the cultures described above and placed upon small, numbered squares of filter paper. The squares were then transferred to Seitz filter pads (about 25 squares could be placed upon a filter pad). The pads were put upon 4-inch inverted pots similar to those described above, and these in turn were set in 10-inch moist chambers. The moist chambers were kept in a specially constructed incubator in which the temperature was maintained constant at $28^{\circ} \pm 1^{\circ}\text{C}$; all the experimental plants were subjected to the same relative humidity by keeping the water in the moist chambers at the same level; constant illumination was also maintained and distributed evenly to all the plants. The latter was accomplished by replacing a portion of the top of the incubator with two plates of glass with an air space between. One of the plates of glass was an opal glass which diffused the light coming from above. The light source consisted of a wooden housing containing six 50-watt Mazda lamps arranged

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in such a way that the light coming from them, when diffused through the opal glass, was uniformly distributed over the shelf upon which the experimental plants were placed.

The prothallia were cut with a pair of iridectomy scissors under the low powers of a binocular microscope. The data on the growth of the prothallium or of parts of it and of the adventitious prothallia were recorded by drawings made with the aid of a Spencer binocular camera-lucida attachment. The surface areas of the drawings were measured with a polar planimeter (Keufel and Esser no. 4240). Since the fern prothallium is composed essentially of a single layer of cells, except in the later stages when

a mid-rib, several cells in thickness, is developed, surface area is a fair index of the mass of tissue present at any time. Increase in surface area was used as an index of growth. Although in the later stages an error may have been introduced because of the presence of the mid-rib, the magnitude of the error was small compared with the individual variations in the experimental material.

RESULTS AND DISCUSSION.—*Normal growth.*—The most extensive experiments on normal growth were carried out on *Pteris aquilina*. Since the early stages of spore germination and establishment of an apical cell in this species are so similar to what has been reported for other Polypodiaceae, the details of these early stages are omitted from the present paper. In the earliest stages used in these experiments, the apex had already cut off a number of derivatives which in turn had exhibited considerable mitotic activity (Dopp, 1927).

Change in form.—In order to study changes in form which might occur as the prothallia enlarged, individual plants, grown under the constant conditions of the experiment, were drawn at intervals over a period of time. In this connection it is important to note that irregular changes in form take place unless the experimental plants are always kept in the same position relative to the light source. These will be described later. In figure 2 is shown a series of drawings of prothallia made at intervals of 3, 6, 9, 12, 16, and 23 days after isolation. The innermost figure in each case represents the prothallium when it was taken from the main culture and isolated on a square of filter paper.

The apparent uniformity in form change in the prothallia shown in figure 2 led the writer to try to ascertain whether the change in form could be expressed in quantitative terms. The relative growth plot (Huxley, 1932) relates the increase of one dimension of a growing organ to another. If growth is regular—i.e., if one dimension (e.g., length) increases

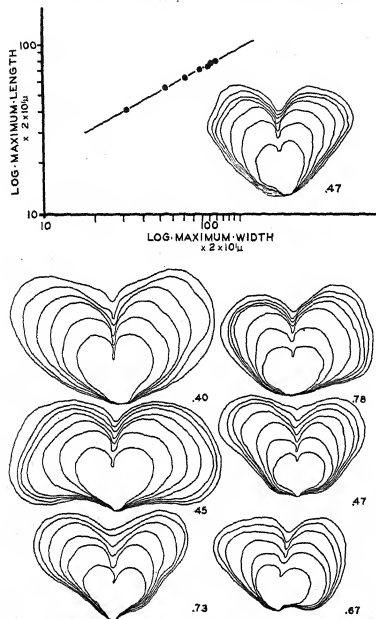


Fig. 1 (above). Relative growth plot for a typical prothallium of *Pteris aquilina*. Camera-lucida sketches of the prothallium at 0, 3, 6, 9, 12, 16, and 23 days following isolation from the germinating culture are shown as well as the value for *k* in the relative growth equation. The detailed growth data are shown in table 1.

Fig. 2 (below). Camera-lucida drawings showing form changes in prothallia of *Pteris aquilina*, grown under constant light, temperature and humidity, at the end of 0, 3, 6, 9, 12, 16, and 23 days after isolation from the germinating culture. The *k* values calculated from the plot of the relative growth equation are shown to the right of each prothallium.

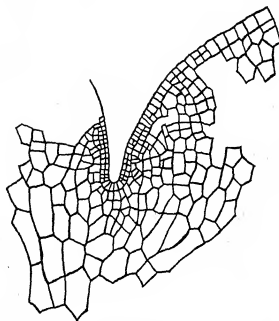


Fig. 3. Camera-lucida drawing showing the arrangement of cells in the apical regions of a typical prothallium of *Pteris aquilina*.

at a constant relative rate with respect to another (e.g., width)—one gets a straight line when one plots the logarithm of one dimension against the logarithm of the other. The equation for the relative growth plot has the general form: $y = bx^k$ where y is one dimension, in this case length; x is the other dimension, here width; b is the value of y when x is 1;

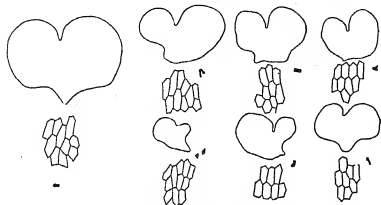


Fig. 4. Relationship between cell size and thallus size. Camera-lucida drawings of small and large prothallia with the smallest and largest cells of each shown.

and k is a constant which determines the value of x for any value of y ; k may be computed from the plot of $\log x$ against $\log y$, and is represented by the slope of the curve. If one gets a straight line when $\log x$ is plotted against $\log y$, then k is constant. By plotting maximum length and maximum width of a typical prothallium logarithmically, the results shown in figure 1 are obtained. The detailed data are shown in table 1. Data for the other plants shown in figure

TABLE 1. Growth data (maximum length and width $\times 2 \times 10^4 \mu$) for a typical prothallium of *Pteris aquilina* (see fig. 1).

Time in days	Maximum length	Maximum width
0	41	32
3	55	55
6	63	73
9	70	90
12	74	100
16	76	103
23	80	112

1, when plotted in the same manner, give similar results. The k values for each are indicated to the right of each prothallium (fig. 2). They range from 0.40 to 0.78.

When measurements of maximum length and maximum width were made by the writer for the single prothallium of *Onoclea struthopteris* which Dopp (1927) was able to follow for 47 days, a good straight-line plot with a k value of 0.58 was again obtained. In *Onoclea*, under the environmental conditions of the experiment, log length is growing constantly 0.58 times as rapidly as log width. Similar regularity in change of form is indicated by the k values that determined in these experiments for *Pteris aquilina*.

Cell size and number.—The largest cells of a fern prothallium are situated toward the basal end; the smallest cells are found in the wings close to the apical notch (fig. 3). If one selects a series of prothallia ranging from small to large, and if one measures the smallest cells and the largest cells of each, one finds that the size of these largest and smallest cells is practically independent of the size of the prothallium (fig. 4). This indicates that small and large prothallia differ from each other chiefly with respect to the number of cells which they possess. This conclusion is substantiated by cell counts and measurements carried out by the writer on Dopp's camera-lucida drawings of different stages of the prothallium of *Onoclea*.

Localized growth.—A closer inspection of figure 3 reveals the interesting fact that cell size increases as one proceeds toward the base of the prothallium and away from the apex laterally on each side. As indicated above, the largest cells are always at the basal end. This observation, together with the data on the change in form of the whole prothallium (fig. 2)

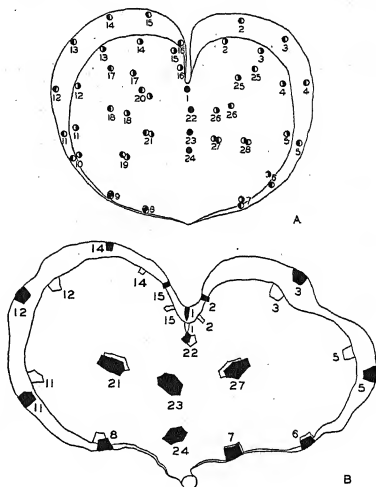


Fig. 5a. Camera-lucida drawings of a prothallium of *Pteris aquilina* at 0 and 6 days after isolation. India ink spots were applied to different portions in order to study growth changes. The spots are numbered to facilitate identification. Circles open at left, original position of spot; circles open at right, final position of spot; solid circles, no movement of spot.

Fig. 5b. Camera-lucida drawings of the prothallium of *Onoclea struthopteris* (Dopp, 1927). The same cells have been identified in two successive growth stages and are numbered in a manner similar to that described above. Open, original position of cell; shaded, final position of cell.

suggested that the greatest growth activity, as represented by increase both in cell number and cell size, was taking place chiefly at the forward end of the prothallium close to the region of the apical notch. In order to test this suggestion, India ink spots were placed upon different portions of prothallia with the aid of a capillary glass rod, and the prothallia were carefully observed over a period of several days. The results of a typical case are shown in figure 5a. The ink spots are numbered on the camera-lucida sketch so that they may be followed. The original position of the spots is shown on the inner sketch. It will be noted that the greatest movement, hence the greatest growth activity, has taken place in the region of the apex; in the basal regions the movements of the spots are very small compared to that of the apical regions. In order to ascertain whether the same kind of phenomenon is illustrated by *Onoclea* (Dopp, 1927), two successive stages of the growth of the prothallium were superimposed by the writer and the same cells identified in each. The results are shown in figure 5b, where the cells have been numbered in a manner similar to that used previously. It becomes at once apparent that the results in *Onoclea* and *Pteris* are strikingly similar.

That the apical region is the center of growth activity may be shown in another way. When an apex of a prothallium is removed by a wedge-shaped cut and isolated, and the remainder cut as shown in figure 6a, the apex shows considerable growth after 6 days; the mid-piece increases only slightly, while the basal piece does not grow at all (fig. 6b). At the end of 14 days, the apex has grown further; the mid-piece has now stopped growing while no growth has taken place at the base (fig. 6c). The relative areas are shown within each piece. Since the mid-piece has stopped growing some time after six days, it is probable that the increase in area during the first six days was due chiefly to an increase in the size of the cells making up the piece and not to an increase in their number. If meristematic cells had been present, one might have expected this piece to keep on growing.

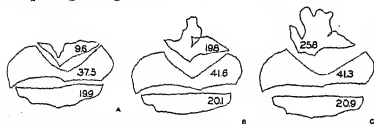


Fig. 6. Camera-lucida drawings of a prothallium of *Pteris longifolia* which has been cut into three parts. The growth of the isolated portions is shown at the end of 0 days (A), 6 days (B), and 14 days (C). The relative areas ($\times 4 \times 10^4 \mu^2$) are indicated within each piece.

Mass relations in normal growth.—As indicated above (fig. 6), when the apex of a prothallium is isolated, it continues to grow, while the basal portions cease growing after several days. The latter develop adventitious sprouts some time later. When

two prothallia of the same size are selected and one of these is cut and isolated, at the end of three days both the intact prothallium and the isolated apex increase their surface area two times (fig. 7). At the end of 6 days the intact prothallium has increased to more than three times its original area, while the

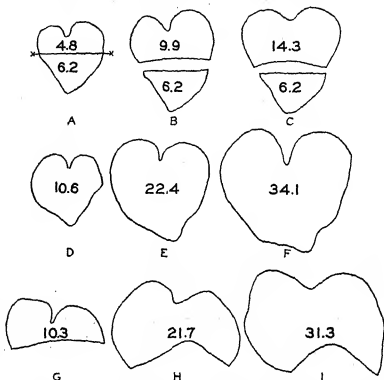


Fig. 7. Camera-lucida drawings showing the relationship between area of the prothallium or of parts of it and growth at the end of 3 and 6 days.

A, B, C—Growth of an apical portion from a prothallium of the same initial size as the intact prothallium, shown in D, at the end of 0 days (A), 3 days (B), 6 days (C).

D, E, F—Growth of an intact prothallium at the end of 0 days (D), 3 days (E), 6 days (F).

G, H, I—Growth of an apical portion of the same initial size as the intact prothallium, shown in D, at the end of 0 days (G), 3 days (H), 6 days (I).

The relative areas ($\times 4 \times 10^4 \mu^2$) are shown within each piece.

isolated apex has increased to the same extent. The basal portion of the cut prothallium has not increased at all over the period of time indicated. The final area of the intact prothallium is almost twice as great as the final area of the isolated apex plus its base, which has not grown at all. This is taken to mean that under normal conditions when the base is attached to the apex, it is contributing materials towards the growth of the apex. What is important here is that while both prothallia have meristematic regions which are equal in size, their masses are unequal, and the increase in size is in proportion to these masses.

That mass is the important thing may be shown in another way. If we compare the growth of the same intact prothallium over the same period of time with an isolated apex of the same initial size, and therefore with a larger meristematic region, we find that both grow to the same size at the end of the same time (fig. 7). The relationship between the mass of a prothallium or of apical portions and the

TABLE 2. Relationship between area of intact prothallia or apical halves of *Pteris aquilina* and the area added at the end of 3 and 6 days. (Each value is the average of at least several plants.) The data are shown graphically in figure 8. (Area $\times 4 \times 10^4 \mu^2$)

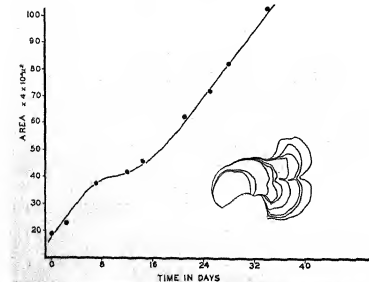
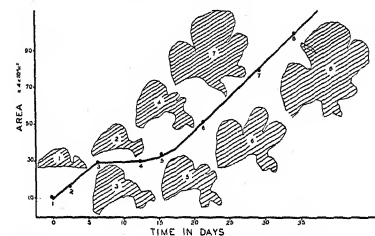
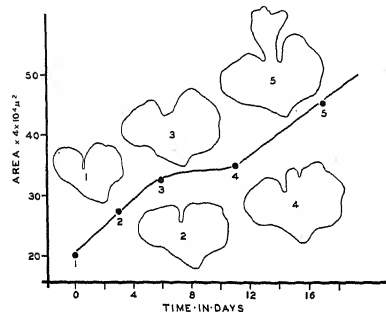
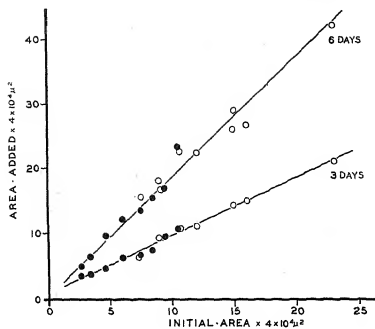
	Original area	Area at end of 3 days	% increase	Area at end of 6 days	% increase
Intact prothallia	7.3	6.6	91	15.8	216
	8.8	9.1	104	18.1	206
	9.1	7.7	85	16.6	185
	10.6	11.0	104	22.1	208
	12.0	11.0	92	22.7	189
	14.8	14.8	100	25.9	175
	15.0			29.1	194
	16.2	15.1	93	26.3	162
	23.0	20.1	87	42.0	182
		Average	94		191
Apical halves	2.7	3.4	122	5.0	185
	3.4	3.6	106	6.5	191
	4.6	4.7	102	9.6	208
	6.0	6.2	103	12.2	204
	7.4	6.8	92	13.8	187
	8.4	7.6	91	15.7	187
	9.4	9.8	104	17.0	181
	10.4	10.8	104	22.4	216
	13.5	10.6	79	23.6	175
	14.0			26.0	186
		Average	103		192

tissue they add at the end of a given time is shown in figure 8, where the closed circles represent data for apical halves, and the open circles, intact prothallia. The detailed data are shown in table 2. The data suggest that we are dealing here with Goebel's "attraction center" concept (Goebel, 1905), where an active region draws materials from other portions of the plant, thus furthering its own growth. Isolating the base from an intact prothallium deprives the active region, in this case the apex, of materials which might have been used for additional growth. In a paper which is to follow, an attempt will be made to link the concept of "attraction centers" to the current growth hormone concept through experiments on fern prothallia.

Effect of reorientation with respect to light.—As pointed out at the beginning of this section, an important factor for the regular growth of fern prothallia is their constant orientation with respect to the light source. Changes in their position result in form changes. Dopp (1927) pointed this out in connection with his controlled growth observations on *Onoclea*. It is also a matter of common knowledge that if the position of a culture of prothallia is shifted, the prothallia reorient themselves in such a way that the apex is displaced towards the light source. Reorientations may also be induced by changing prothallia from their almost vertical position to a horizontal one; the apex now begins to grow up towards the light source, and an interesting type of form

change ensues. Figure 9 shows the form changes and growth curve for a mature intact prothallium of *Pteris longifolia* which had been isolated and placed horizontally upon a piece of filter paper. It may be noted that for a short time the prothallium continues to increase in area at a definite rate, as indicated by the slope of the lower portion of the curve; during this time new cells are being added to the wings, just as in the normally growing prothallium, and cell enlargement is taking place; the apical notch is slowly becoming shallower. During the next interval growth falls off and the cells of the old apical notch begin to grow out. Finally, when the old apex becomes re-established as a new growing center, the growth rate rises almost to the level of the first days after isolation. That initial and final rates are nearly the same may be seen by comparing the slopes of the first and last portions of the curve.

In this connection it is interesting to compare the above condition brought about by reorientation with what one finds in old prothallia (Bauke, 1876; Goebel, 1887, 1905). Goebel observed that old prothallia which had not been fertilized lost their heart-shaped form and became ribbon-like. A close examination of a figure of *Osmunda regalis* published by Goebel (1887) suggests that the ribbon-like character of the old prothallium is due to the fact that after the original prothallium had grown to a certain size, the apex grew out in a manner similar to that described previously. The new outgrowth of the old apex then



grew for a time, whereupon the apex again pushed forth.

Growth of isolated apices.—As shown earlier (fig. 6), when the apical region of a prothallium is cut away from its basal portion and isolated, it continues to grow. The growth curve of such an isolated apex is shown in figure 10. It is apparent that the growth curve obtained is strikingly like that of an intact prothallium which had been placed horizontally with the light source coming from above. Here again, after a time during which there is a slowing down in growth and a reestablishing of the old apex as a new growing center, the new growth rate becomes the same as the initial rate following isolation.

TABLE 3. Relationship between growth of intact and cut apical halves of prothallia of *Pteris longifolia* at the end of 6 days.

	Area ($\times 4 \times 10^4 \mu^2$) at the end of		
	0 days	6 days	% increase
Intact apical halves	8.5	19.3	127
	10.8	29.6	157
	15.6	37.5	140
	18.0	37.3	107
	Average		133
Split apical halves	3.1	7.6	145
	4.7	11.2	138
	7.0	18.0	157
	9.5	24.7	160
	10.6	23.8	124
	11.7	23.6	102
	11.9	25.0	110
	13.3	33.1	148
	Average		135

When an apical region is isolated as above and cut longitudinally through the notch region, each half of the apex behaves as a whole apex—i.e., it regenerates its heart-shaped form (fig. 11). It will be noted that the growth curve has the same form, with the second half of the curve beginning to rise rapidly as soon as the apex is well established again. Growth, here, takes place chiefly on the inner side of the half, the

Fig. 8 (top). Relationship between area of intact prothallia or apical portions and the area added at the end of 3 and 6 days. The detailed data are shown in table 2. Closed circles represent cut apices; open circles, intact prothallia.

Fig. 9 (upper center). Growth curve of an intact prothallium of *Pteris longifolia* reoriented with respect to light. The camera-lucida drawings of the successive stages are shown.

Fig. 10 (lower center). Growth curve for an isolated apical portion of a prothallium of *Pteris longifolia*. Camera-lucida drawings of the successive growth stages are also shown.

Fig. 11 (below). Growth curve for a split apical portion of a prothallium of *Pteris longifolia*. Camera-lucida drawings of the successive growth stages are also shown.

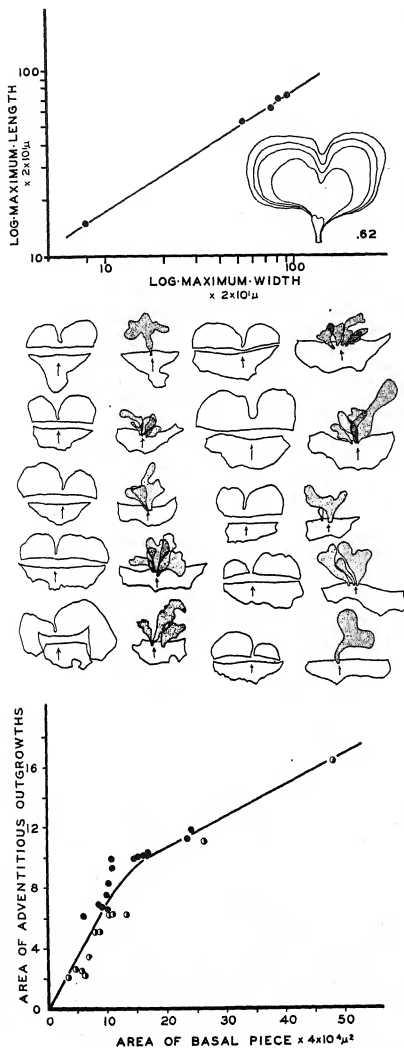


Fig. 12. (above). Relative growth plot for a typical adventitious prothallium of *Pteris longifolia*. Camera-lucida sketches of the sprout at the end of 6, 14, 18, 21,

side which had originally been oriented towards the apex. The other half also carries on the major part of its growth at its inner surface. In the growth of parts of the apex, as with the whole apex and intact prothallia, the same mass relation pointed out previously applies (table 3).

Growth of adventitious sprouts.—When an intact prothallium is cut so that the apex with its actively growing region is isolated from the basal portion, the latter ceases to grow. After about 6 days, however, in the case of *Pteris longifolia*, small outgrowths appear from single cells of the basal part; these grow just as young prothallial filaments do, develop an apical, actively growing region, and often grow into mature prothallia. The number of these adventitious prothallia which may arise from any given basal piece is variable. In general, the adventitious outgrowths tend to be polarized toward the apical end and center of the basal piece; more precisely, they tend to arise towards the position of the apical notch (fig. 13). This polarity, however, appears to be lost the farther the isolating cut is made from the original apical notch.

The adventitious prothallia grow in a regular way under the controlled conditions of the experiment, as is evidenced by figure 12, which represents a relative growth plot for the growth of a typical adventitious outgrowth. The detailed data are shown in table 4.

TABLE 4. Growth data (maximum length and width $\times 2 \times 10^4 \mu$) for a typical adventitious prothallium of *Pteris longifolia* (see fig. 12).

Time in days	Maximum length	Maximum width
6	15	8
14	53	54
18	62	76
21	69	84
27	72	94

As in the case of the intact prothallia, length and width are growing in a perfectly regular, predictable fashion, since k is constant.

Whether one or many prothallia are formed from a single basal piece, the total surface area of the out-

and 27 days are shown, as well as the value of k in the relative growth equation. The detailed growth data are shown in table 4.

Fig. 13 (center). Polarity in the outgrowth of adventitious sprouts from isolated basal portions of prothallia of *Pteris longifolia*. The original cut prothallia are shown to the left of the basal pieces with their adventitious outgrowths. The original polarity of the apical notches is indicated by arrows.

Fig. 14 (below). Relationship between area of isolated basal pieces of prothallia of *Pteris longifolia* and the area of adventitious outgrowths produced at the end of 14 days. The closed and half circles represent data from two different experiments.

growths bears a definite relationship to the surface area of the original piece (fig. 14). The mass relationship described earlier applies, for the amount of growth of any adventitious prothallium at the end of a given time depends on the mass of the piece which gave rise to it. This indicates that the latter is contributing materials to its growth. Previously these materials had been used in the growth of the apical regions which had been attached.

SUMMARY

Quantitative data on the normal growth of prothallia of *Pteris aquilina* under controlled conditions of light, temperature, and humidity have been obtained. These data yield the following conclusions:

The form of an intact prothallium changes in a regular, predictable fashion, since constant values of k are obtained in Huxley's relative growth equation when maximum length and maximum width are plotted logarithmically.

The difference between small and large prothallia is chiefly in the number of cells and not in the size of the cells.

Growth is localized, occurring chiefly at the apical end of the prothallium. Mitotic division is at a maximum in the notch region and close to it; the greatest activity in cellular enlargement occurs directly behind the mitotic region. The cells of the base have reached their maximal size.

In the growth of the intact prothallium or of parts of it which contain meristematic tissue, the amount of growth is dependent upon the entire mass of tissue and not upon the extent of the meristematic region. More basal regions supply materials which are used in apical growth (Goebel's "attraction center" concept).

When prothallia of *Pteris aquilina* or *Pteris longifolia* are reoriented with respect to their light source, changes in form take place. The growth curves of such prothallia are distinctive in that they possess three portions; one in which the growth rate has a definite value, another in which growth almost ceases,

and a final one in which the growth rate attains the initial slope.

Apical regions or halves of apical regions, isolated from prothallia by cutting, regenerate their heart-shaped form. They exhibit the same type of growth curve described above for reoriented intact prothallia.

Adventitious prothallia which arise from isolated basal portions are polarized in their origin, tending to appear close to the cut surface and toward the original apical notch. This polarity is more pronounced the closer the isolating cut is made to the apex.

The total area, and hence the mass, of a group of adventitious prothallia depends on the mass of the piece from which they arise.

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TRANSPLANTATION EXPERIMENTS WITH PEAS¹

F. W. Went

IN A previous paper (Went, 1938) it was pointed out that, while auxin affects many different growth and developmental processes in the plant, the specificity of each reaction must be attributed to the activation of specific factors by the auxin. Evidence for the existence of such specific factors, involved in

root-formation, bud-growth, and leaf-growth, was obtained by cutting off different parts of etiolated pea seedlings; in particular the cotyledons or the roots. By removal of the cotyledons, the further growth of leaves was immediately and completely stopped, and subsequent root formation decreased rapidly, whereas growth in length continued at about half the normal rate. Removal of the roots, but leaving the cotyledons, did not stop leaf-growth or root-formation, but affected mainly longitudinal growth. The case of root formation in particular was worked out in greater detail, and it was shown that auxin by itself does not cause the formation of roots, but only in the presence of another factor. For the sake of

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convenience special terms were suggested for such other specific factors—viz., *rhizocaline* for the root forming factor, which in conjunction with auxin causes formation of roots, *caulocaline*, coming from the roots and necessary for stem elongation, and *phyllocaline*, indispensable for leaf-growth, and in etiolated peas coming from the cotyledon. It was also mentioned that caulocaline moves only through living tissue and has not, so far, been obtained outside the plant. In the previous paper the existence of the calines was deduced from experiments in which their supply was

The grafts were made by cutting the stems obliquely and then forcing the two ends together through a piece of glass tubing 5 mm. long and having about the same bore as the diameter of the stems. If the ends were pressed together, they would hold, and no other precautions were needed to insure good contact. Thus in one experiment 223 grafts out of 470 took within two weeks; in another 130 out of 203. The percentage of successful grafts depended largely on the variety grafted, the person making the graft, and the time of year. Great seasonal variations occurred.

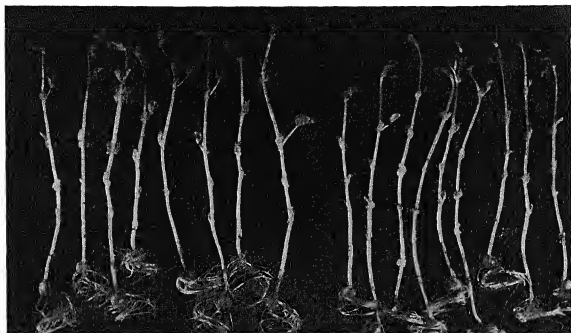


Fig. 1. Peas 18 days after grafting *Daisy* tops on *Daisy* (left) and *Alaska* (right). The graft can be seen in most plants as a thickening (due to the glass tubing keeping the graft partners together) at a distance of about one sixth from the base. In this case the base had no effect on the growth in length of the stem, but only on the leaf development. The third internode of the top is grafted on the first internode of the base, eliminating the second and third nodes bearing bracts.

experimentally cut off. The present paper, on the other hand, follows the opposite procedure and collects evidence for their existence through experiments in which various amounts of each caline are added, while at the same time the food supply is kept as constant as possible.

Technique.—All manipulations were carried out in the same physiological dark room (24°C., 85 per cent humidity) in which the material was growing.

When an etiolated pea stem was cut off above the cotyledons and placed in water, the growth decreased to zero in a very short time. Addition of sugar to the water enabled the stem to continue growth for a few days, though at greatly decreased rate; finally, however, growth stopped completely. So far, no additions in the form of auxin or seed extracts have been found to reestablish growth. By grafting such a stem on a root system with cotyledons, it was possible to restore growth, but only after the graft had "taken"—that is, after a functional connection between the vascular bundles of graft and stock had been established as evidenced by vascular connections through the intermediary parenchyma and callus.

For the sake of convenient handling, the peas were grown individually in 20 cc. glass bottles with about 1 cc. water around the roots, which mostly emerged from the water. Figure 1 shows some grafted peas, the stock consisting of root system, cotyledons, and part of the first internode; the scions or tops were about 4 cm. long at the time of grafting, but had grown into long shoots at the time of photographing.

In most experiments growth of the main shoot was used for measurement of the rootstock effect. But whenever the top bud failed to develop, or was cut off, the growth of a lateral shoot could be used, since growth of both main and lateral shoots seems to be a function of the same set of factors (see, e.g., table 3).

The general growth response of the grafted shoot is as follows. To begin with there is a small residual growth, which practically stops after a few days. From 5 to 12 days after grafting growth is suddenly resumed and rapidly increases until a maximum growth rate is reached. This growth rate may fall off after 1 or 2 days, or it may continue for almost a week. Then the growth decreases to zero again, and this is mostly accompanied by a browning of the tissues at the graft surface. This means that the mean growth

TABLE 1. *Growth of lateral shoots of pea stems in mm./day, as a function of the time after cutting off the main shoots.*

Days after operation	Original length mm.	Growth in mm./day								Final length mm.	Number of shoots grafted
		1	2	3	5	7	9	13	15		
A. Tops only	63.2	1.5	0.6	0.1	0	0.1	-0.1	0	0	65.3	12
B. Tops grafted on base	77.7	3.6	1.9	1.1	0.4	0.	0.	12.7	7	151.2	12
C. Tops grafted on base with insertion of lens paper ...	76.3	2.5	0.9	0.8	0.1	-0.3	0.	0.	0.	80.0	21

rate or the final length of the grafted shoots may vary within wide limits, depending upon the length of time between resumption and cessation of growth. The maximal growth rate, however, has a relatively constant value and therefore was used throughout these experiments, although its determination necessitated daily length measurements of many thousands of grafted peas.

Not only the anatomical examination, but also direct experiments proved the necessity of cellular continuity between stock and graft to make the latter grow in length.

In the first experiment to be described, lateral shoots were used, and their length measured at intervals of from 1-4 days. Five days after decapitation peas were divided into three groups. The first

was no growth whatsoever; then growth of group B was suddenly resumed (in 9 out of 12 plants), but not in groups A or C. Thus the main factor for growth in length was not able to pass from stock to scion if no junction of cells was established (prevented in group C by the lens paper).

Since it is well known that sugar diffuses from living cells to the surrounding medium only slowly, it might be assumed that the growth of the grafted tops could not be resumed because of insufficient sugar supply. To make all groups comparable, the growth of lateral shoots was measured, while a sucrose solution was supplied to the cut end of the main stem by means of a glass cup. To half of the cups auxin was added. In this experiment the graft did not

TABLE 2. *Growth rate in mm./day of lateral buds, growing for 5 days on decapitated plants, which were then cut off and grafted on their own base again. At the time of grafting glass cups were attached to the stump left by decapitation, and these were kept filled with sugar solutions or water, half of them containing 0.6 mg./l indole acetic acid, which solution was renewed every 2 days. Each treatment comprised 10-20 plants.*

Glass cups containing	Sucrose 2%		Sucrose 1%		Water		No cups
	With auxin	No auxin	With auxin	No auxin	With auxin	No auxin	
0-3 days after grafting ...	14.5	16.3	14.8	15.5	10.9	13.2	13.0
3-5 " " " ...	6.4	5.2	5.4	8.1	3.6	5.1	5.1
5-8 " " " ...	3.2	2.9	3.1	4.9	2.0	2.3	5.2
8-12 " " " ...	2.0	1.7	2.5	6.3	0.1	1.1	2.8

group (A) was cut off above the cotyledons, the second (B) was cut off and grafted on its base again, and the third (C) was also cut off and grafted after a strip of lens paper had been inserted between the stock and scion. All three groups were then placed in a 2 per cent sugar solution; the subsequent growth is indicated in table 1. In the first five days after cutting there was still some growth, particularly in groups B and C. Statistical analysis shows that the difference in this initial growth between B and C is not significant, but growth of C, or B and C lumped together, is significantly more than the growth of the non-grafted shoots ($\Delta = 3.2 \pm 1.0$), showing that something in addition to sugar came from the base and diffused across the cut surface, even across the

interfere with the sugar supply. Table 2 shows the results.

It is clear that in all cases the applied auxin slightly retarded the growth of the laterals. Sugar had a slightly beneficial effect, but on the whole the various treatments did not affect the growth rate of the lateral shoots, and thus the initial decrease in growth rate after cutting can not be explained as due to a lack of sugar. The irregular results in the period 8-12 days after grafting were due to the difference in number of shoots which resumed growth. It seems as if the 1 per cent sugar solution had a beneficial effect on the percentage of successful grafts.

In the earlier paper (Went, 1938) it was shown that in the pea seedling the caulocaline mainly comes

TABLE 3. Maximal daily growth rate of main or lateral shoots grafted on their original bases. Only the 5-9 fastest growing shoots recorded in each group, with exclusion of the grafts which had not taken.

	Maximal growth rate in mm./day	Of the grafted tops	Mean	Of the grafted laterals	Mean
(A)	+ cotyledons + roots	41, 36, 33, 33, 32, 26.	33	32, 30, 29, 27, 25, 24, 20, 23.	26
(B)	+ cotyledons — roots	20, 10, 8, 6, 5,	10	16, 15, 13, 13, 12, 12, 11, 10.	13
(C)	— cotyledons + roots	25, 20, 12, 12, 10.	16	8, 5, 4, 3, 3, 2, 2,	4

from the roots, while the cotyledons are a much poorer source. The question arose as to the relationship between the factor lacking in plants with their roots removed and the one passing through the cut surface of a graft. *Alaska* peas were grafted in the usual way, and two days later from group B the roots were cut off, from group C the cotyledons were removed, while the third group (A) was left intact. All three groups were placed with their bases in 2 per cent sucrose solution. Each group was then divided into 2 sets. From one set the stem top was cut off so that a lateral bud would grow out; in the other set the growth of the main stem was measured. The results are summarized in table 3.

For the growth of the main stem the same rule, earlier established, seems to hold—viz., for good growth in length the presence of roots is necessary. This does not hold for the lateral buds; it seems that, without tops or cotyledons, the grafts do not take. On the other hand, it might be that the initial development of the lateral buds is not sufficiently advanced to allow growth in length, and that for this the initial effect of the cotyledons is necessary.

It is a well known fact that in the *Avena* coleoptile a lack of auxin (brought about by decapitation) decreases not only the growth rate but also ultimately decreases the subsequent response to auxin, the phenomenon being called *aging*. This aging, however, occurs only when the coleoptile is connected with the seed; isolated coleoptiles do not age. It is of considerable interest then that the cells of isolated pea shoots do not age.

After pea shoots were cut off, their growth rate fell to zero in the course of two days. One to two days after, growth was resumed in the grafted shoots, and the growth rate reached about the magnitude which it had before cutting (compare e.g. the two top rows in table 7). The maximal growth rate reached by each grafted stem was also independent of the time elapsed before growth was resumed. Table 4 gives the maximal growth rate of *Alaska* tops in mm. per day reached by each of 214 plants grafted in one experiment. They are arranged according to days elapsed between grafting and attainment of maximal growth rate. It will be seen that: (1) In each horizontal column, as well as in the total, there is a very marked bi-modal curve, with peaks at about 5 and 40 mm./day. The first peak of 5 mm./day belongs to the stems in which the graft did not take. Of the total number of 214 peas there are only 2 cases in which it is doubtful whether they belong to the suc-

cessful or the unsuccessful group. (2) The maximal growth rate of the unsuccessful grafts was reached within six days in 64 per cent of cases; whereas in the successful grafts less than 11 per cent of the plants reached their maximal growth within 6 days. (3) If the graft takes, the mean maximal growth rate is the same whether it is reached in 6 or in 12 days. This means that no trace of aging is found. In the other experiments closely similar results were obtained. They enable us to draw some important conclusions in regard to the interpretation of the results. The material is physiologically homogeneous since the distribution curve of the maximal growth rates is strictly symmetrical. It is possible, even in a relatively small group of plants, to distinguish between successful and unsuccessful, grafts if one makes a distribution curve of growth rates. Thus either a stem does not start to grow, but shows only "residual growth," or the stems grow, reaching their maximal growth rate after 6 to 14 days, which growth rate is almost equal to the initial growth rate (before grafting). Thus the temporary limitation in the supply of growth factors imposed by the graft seems to be unimportant. This makes it possible to test the supply of these factors from different sources.

Before turning to the experiments in which different varieties were grafted on each other, another observation must be mentioned. From table 5 it will be seen that if the growth rate in the first two days after grafting is very slow (0-1 mm./day), there is a 75 per cent chance that the graft will take. If, on the other hand, the growth rate in the first two days is more than 3 mm./day, there is less than a 10 per cent chance that the graft will succeed. The percentage of pea stems growing 0-1 mm./day in the first two days after grafting is low in the case of the grafts which do not take and increases rapidly after the 6th day. In the case of the successful grafts matters are exactly the reverse. In the first few days the percentage of stems which do not grow at all is high but rapidly decreases after the 4th day, when they suddenly start to grow at a rate of 10 mm. or more per day. In another experiment the total growth during the first 3 days of the plants which were not going to grow was 18.5 against only 6.3 mm. in the successful grafts.

GRAFTING EXPERIMENTS WITH DIFFERENT PEA VARIETIES.—A number of pea varieties were grown, each pea individually in a 20 cc. glass bottle. As soon as the shoots had grown to a length of about 10-15 cm., the grafts were made. Each shoot was cut off about

TABLE 4. Distribution of maximal growth rates of 214 grafted *Alaska* pea shoots. The figures are differentiated according to the time after grafting that the maximal growth rate of each individual was reached. The last column gives for each group the mean maximal growth rate in mm./day of all successful grafts.

Maximal growth rate in mm./day	0-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	Mean of 20-64 in mm./day
Reached 3 days after grafting ...	5	13	0	0	0	0	0	0	0	0	0	0	0	—
5 days	5	24	0	0	0	0	0	0	1	0	0	0	0	42
7 days	8	6	1	0	0	1	7	10	10	5	2	0	0	39
8 days	3	3	1	0	0	1	7	7	7	3	4	1	1	41
9 days	1	3	2	0	0	4	5	11	4	3	1	0	0	37
10 days	0	0	0	0	1	0	2	4	7	5	0	0	0	40
12 days	1	1	1	1	0	1	1	2	5	9	2	1	0	44
Total	23	50	5	1	1	7	22	34	34	25	9	2	1	40.1 mm./day
	Successful													
	Not successful													

20 mm. above the cotyledon and, if possible, below the first scale. A top of about 40 mm. length was then grafted on. All cotyledonary buds were removed, and every few days the shoots which developed below the graft were cut off before they reached any considerable length and before they started to inhibit the growth of the graft. In all instances the *Alaska* pea was used as reference variety. Each variety tested was grafted on itself and on *Alaska*. *Alaska* tops were also grafted on the variety in question as well as on itself. Tables 6 and 7 give the results of some of the experiments.

Table 6 is a combination of two separate experiments, but since the means of both were very similar, they were combined. The varieties used were *Alaska* and an f_4 of two "raisin peas" (obtained from Dr. H. de Haan), which segregate into *slender*, *tall* and *short* peas. These were easily classified in the seedling stage and the grafts and cross-grafts made. The grafts involving *slender* were difficult to make, since the plants were rather delicate, and only a few succeeded. There was no marked difference between *tall* and *short* in their behavior when used as a base. The tips of the *short* grew less than those of the *tall* on the same base, but these differences were neglected in preparing table 6. It will be seen that both *Alaska* and *tall* and *short* grew much better if grafted on a *tall* or *short* base than if grafted on an *Alaska* base. This was independent of the fact that the growth rate of *tall* and *short* was less than half that of *Alaska* on the same base. Thus the growth rate depended, (1) on the supply of caulocaline from the base and (2) on the specific response of the tip to this amount of caulocaline. In the *slender* plants it seems that both the supply of caulocaline and the response to it were the same as in *Alaska*. This is of interest, since the *slender* seeds could not be distinguished from the *tall* or *short* by their size; and *slender*, *tall* and *short* all had larger seeds than *Alaska* (see table 13). Table 7 gives the data from another experiment, with a few additional figures from an experiment with *Perfection* peas. Although the *Alaska* seeds were the smallest of those used in this experiment, nevertheless in no instance was the growth rate of a top grafted on any base greater than that of the same top grafted on *Alaska*. (The increased growth rate of *Daisy* on *Daisy* was not confirmed in other experiments.) This indicates that the amount of storage food bears no relation to the growth rate of the tops. (For the actual size of peas compare table 13.) On the contrary, in the case of *Little Marvel*, and perhaps of *Perfection*, their bases definitely produced less caulocaline than the much smaller *Alaska* peas. Thus the slow growth rate of *Little Marvel* was due to the interaction of at least two factors. Its caulocaline production was smaller than that of most peas. In addition, it gave a slower growth rate in response to a given caulocaline concentration than did *Alaska*.

A number of other grafting experiments were performed with essentially the same results, so that no

TABLE 5. Percentage of the pea stems which will not grow and of those which will grow, having a certain growth rate at certain periods after grafting Alaska tops on Alaska bases; combination of 2 experiments.

Growth rate in mm./day	0-1	2-3	4-5	6-7	8-9	10-more
Not growing stems (53 plants)						
In 1st and 2nd day after grafting	31%	23	19	21	4	2
3rd and 4th day	19	30	25	15	9	2
5th " 6th "	23	30	30	9	6	2
7th "	46	23	15	7	7	0
8th "	61	31	8	0	0	0
9th "	92	0	0	8	0	0
Growing stems (50 plants)						
In 1st and 2nd day after grafting	83%	13	2	2	0	0
3rd and 4th day	67	10	8	2	2	11
5th " 6th "	10	8	8	11	5	58
7th "	25	4	3	0	0	68
8th "	11	4	3	0	7	75
9th "	7	0	3	3	3	84

TABLE 6. Maximal growth rate in mm./day of the tops after grafting three pea varieties on each other.

Top of	Alaska		Tall and Short		Slender	
	MEAN	Number of plants	MEAN	Number of plants	MEAN	Number of plants
Alaska	29.2±1.4	15	12.9±0.5	24	29.6±2.4	5
Tall and Short	40.9±1.3	11	18.7±1.1	16		
Slender	31	2			32	1

further data need to be given. We may now consider the facts thus far presented.

It is possible to differentiate between several of the factors involved in the longitudinal growth of the pea stem. Two sets of such factors must be clearly differentiated. In the first place, we know that auxin, which is produced in the terminal bud and youngest leaves, is necessary for this growth. An analysis of de Haan and Gorter (1936) has shown that in the case of *slender* peas (the "slender" used in this publication) the large growth rate must be ascribed to a low rate of auxin destruction, which is much larger in the relatively short *tall* and *short* peas. Thus a part of the growth differences of peas may be ascribed to variations in the available auxin. This is also indicated by the experiments of Michener (1937), in which it was shown that there is a much larger auxin destruction in *Perfection* than in *Alaska*. This is in complete agreement with the conclusions of van Overbeek concerning the causes of dwarf growth in corn (1935, 1938). The effects of auxin production and auxin destruction are confined to the tip and growing region; for each grafted top they are "autonomous." The differences in the growth rate of tops of different varieties grafted on bases of the same variety must be connected with these auxin relations. But the foregoing experiments have shown that in addition to these autonomous top-auxin relations a factor (or

factors) supplied by the base, to which the name caulocline has been given, determines the growth rate of the grafted tops. With a given variety of tops a decreased (autonomous) growth response may be linked with an increased (*tall* and *short*), a decreased (*Little Marvel* and perhaps *Perfection*), or an unchanged caulocline production of the base relative to that of *Alaska* (*Hundredfold*, *Stratagem*, *Daisy* and *Morse's* 200). On the other hand *slender* and *Alaska* both have about the same caulocline supply and top-response.

There is another obvious conclusion already mentioned in an earlier paper (Went, 1938). Other plants in addition to the pea varieties may differ in their caulocline production. Thus the dwarfing effects of certain *Citrus* or *Pyrus* rootstocks on the grafted tops may be due to causes similar to those in the experiments described above; in general the rootstock-scion relation receives a theoretical foundation when we realize that special growth factors may move from stock to scion.

LEAF AND STIPULE GROWTH.—In the course of the experiments described above it soon became clear that not only stem elongation was affected by the stock but that the development of practically every organ of the growing top was influenced in one way or another. The basis for an intelligent interpretation of such effects may be found in an earlier paper (Went,

TABLE 7. Mean maximal growth rate of shoots of different pea varieties which were grafted on bases of the varieties mentioned in the left-hand column. Under each figure the number of successful grafts, from which the mean was calculated. Upper row, growth rate of the various peas in the 2-day period just preceding the grafting, expressed in mm./day.

Growth rate in mm./day of intact plants		48.0	25.3	22.1	19.0	12.5	15.2	16.8
		Alaska	Perfection	Hundred- fold	Stratagem	Little Marvel	Daisy	Morse's 200
Base of	Top of							
Alaska	Mean Number	42.7±0.9 30	15.3±0.6 21	14.3±0.9 9	17.3±0.8 19	13.0±0.7 9	13.6±0.9 12	15.4±0.7 14
Perfection		37.7±1.0 24	13.5±1.2 10					
Hundredfold		42.4±1.9 13		13.3±0.8 9				
Stratagem		43.0±1.5 16			17.9±1.6 8			
Little Marvel		34.0±2.1 7				9.6±0.7 14		
Daisy		40.4±2.4 13					15.7±0.6 8	
Morse's 200		40.1±2.7 10						15.6±0.5 11

1938), in which it was shown that leaf development on etiolated pea shoots continues in darkness only as long as the cotyledons are present. It was concluded that the cotyledons store a specific leaf growth substance, phyllocaline, which is formed in leaves in the light.

The darkroom conditions under which the peas were grown for the grafting experiments did not permit of the synthesis of phyllocaline and this simplified the interpretation of the results. It was found that in none of the grafted shoots did any leaf growth

or development take place before the graft union was established. No attempt was made to follow the course of the leaf growth, but at the conclusion of each experiment, 14-20 days after grafting, the leaf length, leaf width, petiole length, stipule length, stipule width, and tendrill length of all leaves on the successful grafts were measured.

In table 8 the mean sizes of the first (oldest), second, third, and fourth leaves on a few of the successful peas are given, the numbers ranging from 7-19 in different groups. There was extensive variation in

TABLE 8.

	Leaf			Petiole	Stipule			Tendrill
	Length	Width	Surface		Length	Width	Surface	
Daisy on Alaska								
First leaves	7.9	7.6	60	5.8	7.3	5.3	39	2.3
Second leaves	8.5	7.6	65	6.1	9.6	7.1	68	4.1
Third leaves	8.5	9.0	76	7.7	9.1	6.7	61	6.2
Fourth leaves	8.2	7.7	63	8.0	9.7	6.0	58	10.0
Daisy on Daisy								
First leaves	8.1	7.9	64	5.7	7.6	6.1	46	1.9
Second leaves	8.6	8.8	75	7.9	11.3	9.8	111	4.2
Third leaves	9.4	9.9	93	10.6	12.6	10.0	126	5.7
Fourth leaves	13.7	13.4	182	16.3	14.2	10.5	149	11.6
Alaska on Alaska								
First leaves	8.5	5.5	47	6.5	5.5	3.5	19	3.2
Second leaves	8.2	5.6	46	6.5	7.1	4.1	29	5.0
Third leaves	7.8	4.9	38	10.2	6.8	4.2	29	7.3
Alaska on Daisy								
First leaves	8.6	4.6	40	8.6	5.3	3.9	21	3.1
Second leaves	9.3	5.2	48	12.1	7.6	4.8	36	5.4
Third leaves	10.0	5.7	57	17.9	9.1	5.2	47	12.2

divided into two groups, according to their leaf size. These were used to graft *Alaska* and *Daisy* tops on. Table 9 shows that when for some environmental reason a certain individual produces a small leaf, it is as good a source of phylloclaine as a large-leaved plant of the same variety.

It is evident that almost without exception *Alaska* tops grafted on a base of a given variety gave better leaf, stipule, or petiole development than when grafted on *Alaska* itself; the same effect was noted when other varieties were used as top. There is no evidence that better development occurred in the auto-transplanta-

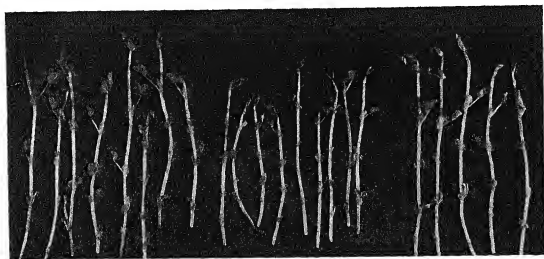


Fig. 2. *Daisy* tops, 18 days after grafting on *Tall* and *Short* (left), *Perfection* (center), and *Morse's 200* (right). Note the deficient leaf development and the shortness on *Perfection* bases, and the large stipules on *Morse's 200*.

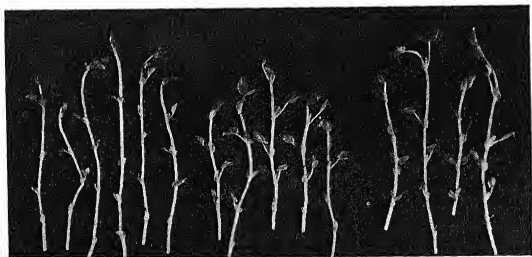


Fig. 3. *Little Marvel* tops, 18 days after grafting on *Alaska* (left), *Stratagem* (center), and *Tall* and *Short* (right). Only slight leaf development on bases of *Alaska*.

Figures 2 and 3 give an impression of the differences in leaf size, etc., of the tops of *Daisy* and of *Little Marvel*, which had been grafted 18 days before on different stocks. It is clear that *Perfection* or *Alaska* as base were far inferior to *tall* and *short*, *Stratagem*, or *Morse's 200* with respect to leaf development, although no remarkable differences in length were induced by the various bases. It was even rather easy to recognize the base upon which a given top had been grafted by the development of the leaves, stipules, etc., in the successful grafts. The results of two large experiments are given in tables 10 and 11, in which the values for leaf and stipule surface, as well as petiole and tendril length, are given. The surface was obtained by multiplying the mean length by the mean width of all leaves measured in the particular group. This does not of course give an absolute but rather a comparative value.

tion, where perhaps a better fit of the grafted parts might be expected. Another point should also be noted. Some varieties responded better than others to differences in stocks; thus *Daisy* and *Little Marvel* were especially suited to detect differences in leaf growth, whereas *Alaska* was rather unresponsive.

To make a simpler comparison of the differences possible, table 12 summarizes the results from tables 10 and 11 by expressing the effects of a given base in terms of the similar effect of the *Alaska* base, as 100.

It has just been mentioned that some varieties used as top give much better responses than others. Partially to eliminate the differences in relative effect of the various bases, caused by the use of many different tops as detectors, the figures were corrected, particularly for the tops of *Stratagem* and *Little Marvel*, the former giving a consistently smaller response (68-93

TABLE 11. *Leaf surface, stipule surface, petiole length, and tendril length of the largest leaf developed after 2-3 weeks when this pea was grafted on the variety named in the first column. Upper figure, leaf surface; upper middle figure, stipule surface; lower middle figure, petiole length, lower figure, tendril length.*

Top of	Alaska	Daisy	Stratagem	Little Marvel
Grafted on base of				
Alaska	50 33 10.6 9.4	75 56 7.9 8.7	114 70 10.0 8.4	65 40 7.2 7.8
Daisy	70 48 13.9 10.3	160 90 11.1 9.9	154 85 13.3 8.8	155 102 12.1 13.0
Stratagem	61 39 10.2 6.2	100 67 8.5 6.5	167 71 10.8 8.6	167 89 12.6 12.2
Little Marvel	84 45 8.0 7.1	102 47 8.0 10.1		148 70 15.5 12.5
Perfection	70 45 9.7 7.7	84 49 7.0 8.1	109 50 7.7 7.8	
Morse's 200	48 29 5.2 5.7	128 119 10.6 9.4		
Tall and Short	60 37 13.0 7.0	144 129 12.2 8.8		143 91 14.1 12.4
Slender	35 25 6.2 4.0			

per cent), the latter a larger response (114-159 per cent) than the average. It is of interest to note that if in any variety the leaf growth response is increased or decreased, it is increased or decreased to about the same extent for petiole and stipule growth.

It is clear that the responses of the various tops are rather consistent if one considers how "derived" the figures are. The means have been calculated, and the effect expressed in + or - in comparison with *Alaska*. If we combine these results with those obtained from the longitudinal stem growth, table 13 results. In this table the peas are arranged according to the dry weights of the seeds, and the table makes it quite clear again that the effects are not due to one general nutritional factor. The four measured values—leaf surface, stipule surface, petiole length, and stem length—are affected differentially; one base increases one process but decreases another, another base increases both, etc. There is only rough parallelism between dry weight of pea and its general effect on growth. This means that the growth of each of these organs is affected by a different factor or set of factors. The elongation of the stem has

been traced to the effect of caulocaline, coming from the root system. The growth of the leaves is due to phyllocaline, stored in the cotyledons (Went, 1938). Now it is possible that stipule growth requires the same factor, and that in some way the distribution of the available phyllocaline over leaf and stipule is affected by the stock. So far there are no experiments available to distinguish between these possibilities, and the hypothesis of a special stipule-caline is premature, but in either case we need the assumption of a specific effect coming from the cotyledons. Finally, the petiole growth might be considered as brought about by the combined effects of auxin produced in the leaf blade and caulocaline coming from the stock. The larger the leaf blade, the more auxin would be available, and the longer the petiole would grow. The same effect might be caused by an excess of caulocaline. Indeed, if we add the effects of the base on leaf surface and stem length (table 13), we get the proper sign for the effect on petiole length with the exception of *Morse's 200*. The figures in table 10: *Alaska* grafted on *Morse's 200* are, however, unreliable being the mean of only 2 successful grafts. There

TABLE 12. *Different varieties of peas are grafted either on Alaska or on the variety named. Then the response in leaf, stipule, or petiole-growth is expressed as % of response of the same top on Alaska. Figures are corrected for relative responsiveness of varieties. Each figure is the mean of the comparison between about 2 x 10 successful grafts.*

Base of Top of	Perfection			Daisy			Stratagem			Little Marvel			Morse's 200		
	Leaf	Stip.	Pet.	Leaf	Stip.	Pet.	Leaf	Stip.	Pet.	Leaf	Stip.	Pet.	Leaf	Stip.	Pet.
Alaska	152	148	100	152	158	143	133	128	104	183	148	83	144?	119?	53?
	111	88	137	144	175	133	176	143	154	133	101	137	152	172	140
Daisy	112	87	89	213	161	141	133	120	108	136	84	101	171	213	134
				170	225	161									
Stratagem	116	86	93	162	146	160	176	122	130						
							184	196	151						
Little Marvel				178	190	125	192	166	131	170	131	161			
										116	66	72			
Perfection	104	123	129												
Morse's 200													139	239	101
Mean	119	106	110	170	176	144	166	146	130	148	106	111	151	186	107
Effect	+	?	0	0	++	++	+	+	+	+	0	0	+	++	0

are additional reasons for considering petiole growth to be due to the same factors as stem growth. In exceptional cases the apical bud of a pea suddenly ceases to develop. In such cases either a lateral bud starts to elongate, or the petiole of the most apical growing leaf grows out to exceptional length. As long as this petiole grows, no lateral buds develop. Petioles as long as 50-70 mm. have been observed under such conditions. This means that the factors necessary for elongation of main or lateral stems are temporarily diverted towards the petiole, which then may grow to exceptional dimensions.

In some preliminary experiments in which peas were grown in light of different colors, the conclusion was also reached that leaf growth, stipule growth, and stem growth were differentially affected, which supports the conclusion that leaf, stipule, and stem growth are governed by different factors, which were formed or rendered active by different wave lengths.

When the leaf growth in the experiments described above was increased or affected in any way, leaf length and width were increased in the same proportion, so that the leaf form was not changed. The stipule shape was also unaffected by any of the rootstocks affecting stipule size. This means that in the peas investigated, leaf and stipule form were specific characters, not modified by external or internal factors. Even without changes in the shape of the various organs of a grafted plant, the general appearance of a given variety could, however, be definitely changed by alteration of the relative sizes of internodes, leaves, etc.

In connection with the caulocaline supply from the cotyledons another possibility must be borne in mind. The auxin produced in the apical buds is derived from a precursor, which comes from the cotyledons. Although the precursor is water soluble (Skoog, 1937),

can move across the cut surface of the graft, and may be transformed into auxin in the top, no growth is possible before the caulocaline supply is renewed. As soon as the junction of the tissues is established, caulocaline is available and growth is resumed. Thus it is conceivable that not caulocaline but the auxin precursor supply limits the growth rate of the grafted tops. Ether extractions of such tops, however, did not reveal a correlation between auxin content and growth rate, so that a factor other than auxin is limiting top growth in the grafted peas.

The data discussed above give a good illustration of a new use of transplantation experiments. So far they have furnished evidence for the immutability of species in direct contact with others, translocation of alkaloids, viruses, etc., mutual effects of epidermis, cortex, and central cylinder in the development of plant form, cell polarity, individuality of cells and organs in the utilization and transformation of nutrients, acquired immunity, etc. But so far as I am aware, they have not been used as yet to determine which developmental processes in the plant are hormone-determined—that is, between which parts functional correlations exist. In this respect a closer analysis of the periclinal chimæras which already yielded so many important clues as to developmental interrelations must furnish a remarkably rich source of information concerning hormonal relations in the development of tissues. Haberlandt's analysis (1927) of the *Crataegomespili* indicates some of the possibilities.

Another question which comes up is why the known effects of the rootstock on the development of the scion are practically limited to general effects on the size of branches and why no effects on leaf size, etc., are known. The answer to the first part of this question has been given before; the size of branches

is determined among others by the caulocaline coming from the rootstock. But the leaf size depends on the production of phyllocaline, produced in the leaves of the scion itself. Thus leaf size is not determined by the rootstock. In peas the situation is different, since in the experiments described the rootstock comprised the cotyledons and hence supplied the phyllocaline, which in the dark room could not be synthesized by the scion.

Of course it would be most important to link these differences in the supply of hormones by the various pea varieties with the genetic factors determining their development. Unfortunately only a few genes involved in the growth of peas are known, and the genes known have been investigated (*slender* versus *tall* and *short*) (de Haan and Gorter, 1936).

the initial rate. If different pea varieties were used either as stock or as scion, then the growth of the grafted tops depended (1) upon the specific reactivity of the top and (2) upon the supply of certain growth factors, the calines, from the base. Stem elongation, leaf growth, stipule growth, and petiole growth were differentially affected by the pea varieties used as rootstock, so that the conclusion is reached that each of these processes is affected by a different factor or set of factors. These factors, which come from the base and move through living tissues only, have provisionally been placed together in a new category of plant hormones, the calines. Further investigation must reveal their actual nature.

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TABLE 13. Summary of effects of base with Alaska as reference.

Variety	Weight of one pea seed in mg.	Stem length	Petiole length	Leaf surface	Stipule surface
Alaska	219	0	0	0	0
Perfection	222	—?	0	+?	0
Little Marvel	235	—	0	+	0
Hundredfold	255	0	+?	0	0
Tall and Short	279	+	+	++	++
Slender	279	0	—	—	—
British Empire	296	0	+?	0?	0?
Stratagem	300	0	+	++	+
Morse's 200	326	0	0	+	++
Daisy	379	0	+	++	++

All data presented in this paper furnish further evidence for the existence of a new class of plant hormones, the calines, postulated in an earlier paper (Went, 1937). Especially the fact that they move through living tissue only places them in a category by themselves: no other factors thus far known behave in this way. This also excludes the possibility that these factors are nutrients, such as special forms of sugars, since these would be extractable. Moreover, Bonner and Axtman (1937) were unable to find any nutritive medium, synthetic or complex, which could exert the same effects as these calines.

SUMMARY

A number of elaborate experiments have been carried out in which 4 cm. tops of etiolated pea shoots were grafted on root systems (with attached cotyledons) of the same or other peas. It was found that until actual union of the grafted peas through junction of the tissues by regenerated vascular bundles had occurred, no growth of the grafted tops, either of stems or of leaves, took place. After the junction was completed, growth was renewed at approximately

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ENDOSPERM AND PERISPERM OF COFFEE WITH NOTES
ON THE MORPHOLOGY OF THE OVULE
AND SEED DEVELOPMENT¹

William G. Houk

It is generally agreed among botanists that the nucellus is homologous with the sporangium of the lower vasculares, and the integuments are regarded as structures distinctive of more recent vegetation. Plants having ovules with two integuments are rather common (most monocotyledons and dialypetalous dicotyledons); those with one integument are less numerous (in most of the sympetalous dicotyledons); and naked ovules occur only infrequently (Santalaceae, Loranthaceae, Balanophoraceae, Olacaceae, saprophytic Gentianaceae, and species of *Crinum* (Wettstein, 1935)). Since the reduction of the integument is an indication of specialization and evolutionary advancement, its extreme expression is not surprising in parasitic or saprophytic plants or in a family as advanced as the Rubiaceae. At least one genus of the Rubiaceae has ovules with no integument (*Houstonia*), and the genus *Coffea* appears to have ovules in which the nucellus and integument give no indication of being separate structures. As in many other Rubiaceous genera, coffee has an obturator, a structure which grows out from the placenta. The origin of the obturator differs from that of the aril, which arises from the funiculus, or the caruncle, which is an outgrowth of the integument.

Schleiden (1837) seems to have been the first to discuss the nucellus of the Rubiaceae; he stated that it is naked—that is, without an integument. Warming (1878) refuted this statement, apparently successfully, when he said that the presence of a slender micropyle, surrounded by a massive integument would account for the earlier reports of the absence of an integument, because a slender micropyle might easily be overlooked. That there is more to the problem than simply finding a micropyle has been shown by later work which demonstrated that the absence of that structure is no criterion of the nakedness of an ovule. Moreover, the unusual nature of the Rubiaceous ovule has been indicated in Lloyd's reporting a naked ovule in *Houstonia* and the intimate relation and similarity between the integument and nucellus in certain other genera.

During an examination of the ovules of some of the Rubiaceae evidence was found supporting the interpretation of the integument-nucellus of Lloyd (1902) for other members of the family, rather than von Faber's (1912) interpretation or the one occurring at first to the present writer for coffee (Houk, 1936);

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see below. Another morphological feature of great practical importance which is closely related to the integument-nucellus problem is the presence of perisperm and the evanescent nature of the endosperm. For many years the nutritive material surrounding the embryo of coffee has been referred to as endosperm; however, as will be shown presently, only a few cells of endosperm are formed, and these soon degenerate. As a rule a seed having perisperm has some endosperm also, but this is not true of the coffee seed.

MATERIALS AND METHODS.—The coffees used in this study were *Coffea robusta* L. Linden and *Coffea arabica* L. var. *typica* Cramer, the latter being a cultivated variety known in Brazil as "nacional" or "commun." Other varieties of *C. arabica* showed no essential variation; consequently, detailed examination was restricted to *C. arabica* var. *typica*. Observations were made not only on normal flowers but also on the small abortive flowers known as "estrelinhas" (Portuguese) or "sterretjes" (Dutch)—here called starlet flowers. The starlets have an extremely short corolla tube with abbreviated fleshy lobes, and the stamens and ovary are abnormally small. The ovules often have a megaspore mother cell, but it is most unlikely that embryo sacs ever develop. Since some of the starlets are more completely aborted than others, all types, from much reduced to normal flowers, can be found.

The paraffine method was used to prepare the material for study. Both Heidenhain's iron alum hematoxylin, and the erythrosin-crystal violet combination described by Jackson (1926) were used for staining, the former being used for cytological details in the embryo sac.

The investigation was carried out as a part of the work in organizing a Department of Botany in the Instituto Agronomico, the central agricultural experiment station for the state of Sao Paulo (Brazil).² The Instituto is located at Campinas, a city of nearly 100,000 population, in the heart of the district which produces the so-called "Santos" coffee. The Instituto at Campinas has a 600-acre farm just outside the city. The station is under the direction of Dr. Theodureto de Camargo, whose work on the physiology and nutrition of coffee is widely known.

GENERAL MORPHOLOGY OF THE COFFEE FLOWER.—The flowers of *Coffea* are white, regular, and perfect and are disposed in axillary glomerules; they are only rarely terminal. The completely inferior ovary has two one-seeded locules and is surmounted by a

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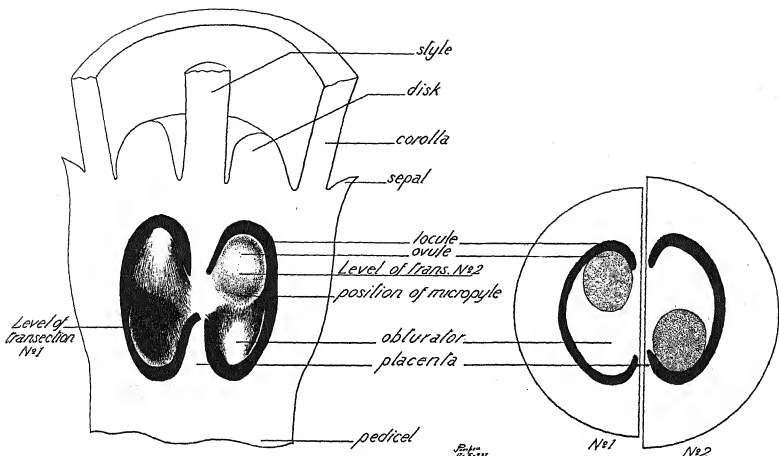


Fig. 1. (Left) Longisection of coffee flower. Stamens and corolla lobes not shown. The obturator leaves the micropyle free on one side but covers the entire ovule on the other side. (Right) Transsections at levels indicated in the longisection.

thick ring-like disc or nectary. The sepals, in most species, are represented by minute teeth. In *C. arabica* var. *typica* and *C. robusta* there are five petals and an equal number of stamens attached high up in the corolla tube. Von Faber (1912) has described the general morphology of the flower, but for convenient reference a diagram in longi- and transection is shown in figure 1. The micropyle is always found at the lower end of the ovule and on the side not covered by the obturator.

DEVELOPMENT OF PERISPERM.—The folded nutrient tissue surrounding the embryo is produced by the enlargement of the nucellus-integument and the elongation and enlargement of the funiculus, as shown in the series of diagrams, figure 2.

Figure 2a shows a very young ovule and the obturator. By growth upward, and toward the placenta, the ovule crowds and eventually obliterates the obturator, and remains of which are marked "X" in figures 2b, c, and d. In d the obturator has practically disappeared, and in e it is gone. Figure 2g is the reverse of f; g is larger because of the distinct tapering from one side of the seed to the other. By the time the seed has attained a length of about one millimeter (the stage shown in f and g), a deep hollow (marked "con" in figure 2g) has formed in the enlarged upper part of the funiculus. This concavity enlarges, and through the growth of the tissues on its sides the ovule proper is eventually covered (see the transection of the locule and the nearly mature seed in fig. 2h). The tissues of the upper part of the funiculus and of the chalazal end of the

ovule are identical in appearance. Figure 2i, a transection of a nearly mature fruit, shows the position of the seeds in their locules.

It is evident from the development of the ovule that the folded nutrient tissue around the embryo is perisperm derived from the body of the ovule and the elongated and enlarged funiculus.

Only in the last few years has a start been made on the genetics of coffee, but now that improvement programs are under way, it is highly important to recognize that the object of the improvement—namely, the bulk of the seed—is of the parental generation (perisperm) and not of the new generation as is the plantlet in the seed. The little endosperm that is formed is notable both because of its paucity and evanescence.

DEVELOPMENT OF THE ENDOSPERM.—The almost impermeable tissues of the ovary wall and the fact that each flower has but two ovules, and sometimes only one, make the satisfactory preparation of material particularly difficult. The former difficulty can be met by cutting off both ends of the ovary before fixing and by leaving the material in melted paraffine a long time. It is even more satisfactory to dissect out ovules that are large enough to be manipulated, for then the fixing agent and the paraffine can enter easily.

Von Faber (1912) described the gametophytes of *Coffea* in some detail, reporting the embryo sac as of the normal type. There is little doubt that the polar nuclei fuse, but the present writer was unable to confirm or question von Faber's statement concerning double fertilization.

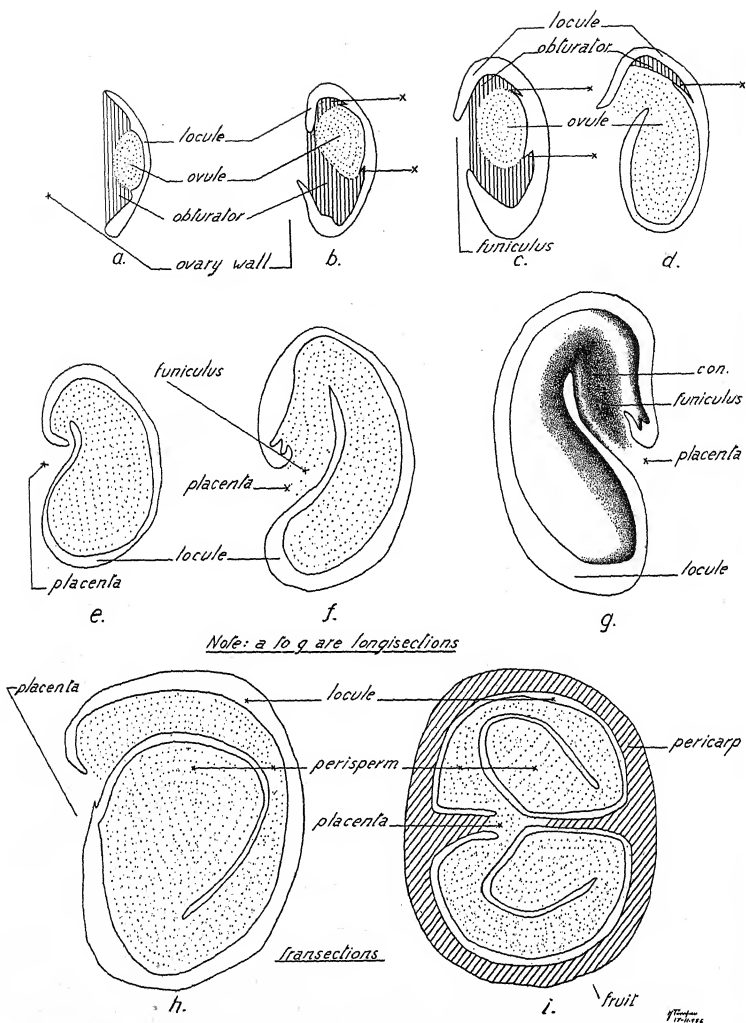


Fig. 2. a, ovule partly embedded in the obturator; b, c, d, and e, gradual obliteration of obturator by the growing ovule (obturator marked "X"); g the reverse of f, is larger, for the seed tapers distinctly from one side of the seed to the other. "Con" in g, concavity formed by the enlarging upper part of the funiculus; the ovule proper is eventually wrapped in this enlarged part of the funiculus (h); i, transection of a nearly mature fruit showing position of seeds in the locules.

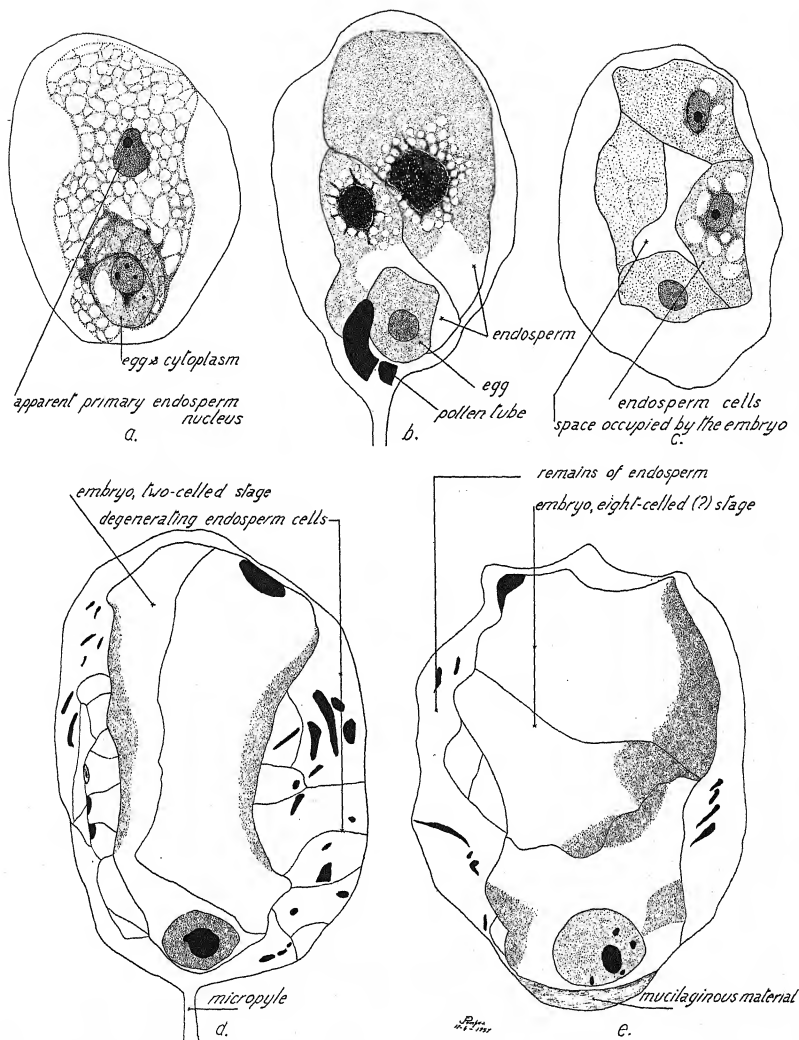


Fig. 3. a, fertilized egg and primary endosperm nucleus; b, fertilized egg, remains of pollen tube, and 2-celled stage of the endosperm; c, four of eight (?) cells of endosperm; the opening marks the location of the bladdery 2-celled embryo; d, two-celled embryo and rapidly degenerating endosperm cells; e, 8-celled embryo and two badly crushed endosperm cells.

After fertilization the egg enlarges somewhat and then undergoes a rest period, during which the synergids disappear and the apparent primary endosperm nucleus divides a few times. Remains of the antipodals, especially of the middle one, persist for some time.

Figure 3a shows the fertilized egg and the primary endosperm nucleus. The large vacuoles are characteristic and persist sometimes until several cells are produced. In young stages these vacuoles are rather puzzling, for at that time they are very small and full of a substance that stains blue with crystal violet. Von Faber (1912, p. 81) states that the small bodies are the remains of the nucleoli of the degenerating daughter cells of the tetrad (which, he says, is coenocytic); but the subsequent development of the "granules" into vacuoles is not in accord with this view. Figure 3b shows the fertilized egg, remains of the pollen tube, and the two-celled stage of the endosperm. The endosperm is not always strictly of the cellular type, for one embryo sac was observed in which four endosperm nuclei were present with the walls not yet formed; the spindle fibers were clear between the nuclei which had just completed their divisions. Figure 3c shows four of eight (?) cells of the endosperm. The opening in the center marks the location of the embryo, which, at this stage, consisted of two large bladdery cells. Figure 3d represents the two-celled embryo and the rapidly degenerating endosperm cells. Two badly crushed endosperm cells can be distinguished in figure 3e where the embryo consists of eight (?) cells. The lines at the bottom of the figure represent the beginning of the formation of a layer of mucilaginous material that eventually surrounds the embryo.

The changes in the embryo occurring subsequently to those shown in figure 3e consist of the accumulation of more cytoplasm in the cells and the laying down of cross walls. Up to about the fifth month after fertilization, in the Campinas region, the embryo remains as an undifferentiated spindle-shaped mass of cells. Cotyledons are then differentiated, but the epicotyl becomes visible only after the seedling is well along—that is, about eight centimeters high.

Records were kept in an effort to correlate the age of the ovules with the stage of development of the embryo sacs, but as often as not the sac in a twenty-day ovule was at the same stage of development as that in an eight- or twelve-day ovule.

DISCUSSION.—In 1902 Lloyd pointed out the intimate relation and the difficulty in distinguishing between nucellar and integument tissues in certain Rubiaceous genera. He called attention to the difficulty, in such cases, of delimiting the nucellus as it is ordinarily defined. Although Lloyd did not study *Coffea*, his report for certain other genera fits this genus. The micropyle of the coffee ovule is extremely slender, and there is no differentiation of cells

indicating a separation into integument and nucellus. It is difficult to understand the basis of von Faber's (1912) statement that a massive integument and a weakly developed nucellus are present. The cells around the embryo sac are crushed as it grows, and their different appearance is caused by this alone. It seems clear that von Faber's interpretation is not tenable, and since the nucellus can hardly be considered to be suppressed, only two other interpretations of the tissues surrounding the embryo sac appear to remain: (1) the suppression of the integument and the separation of the nucellar cells to form a pseudomicropyle (Houk, 1936), and (2) the interpretation of Lloyd as mentioned above—viz., that both integument and nucellus are present, but they are so much alike and so intimately associated that they cannot be distinguished as separate structures.

The writer's observations have led to his acceptance of Lloyd's interpretation, though the structure of the ovules in the starlet flowers, and certain features in others, do offer considerable support for the view that the integument is suppressed. Von Faber (1912) stated that the starlet ovules lack an integument—a view with which the present author cannot agree; the difference between the starlet ovules and the others is only in the lack of a definite micropyle. There is, however, in many starlet ovules a strong tendency for the tissues of the ovule to split apart in the region of the megaspore mother cell.

It is suggested that the lack of differentiation of tissues of the ovule may be caused by the presence of the extensively developed obturator; in the resulting uniform tissue environment all the cells develop in nearly the same manner.

SUMMARY

The ovule of *Coffea* is accompanied by an extensively developed obturator. There appears to be no distinction between the tissues of the nucellus and those of the integument, the two being intimately fused.

Endosperm production is scanty, and the few cells produced are soon crushed by the developing embryo. As a rule, walls are laid down immediately after the division of the endosperm nucleus, but sometimes wall formation is delayed for a time, or perhaps in some instances is omitted. The mass of tissue around the embryo is perisperm and thus belongs to the parental generation—a point of great importance in breeding work.

The embryo is of the undifferentiated type, consisting of only a mass of cells until about the fifth month after fertilization.

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EFFECT OF ENDOTHIA PARASITICA ON CONDUCTION¹

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DISCOLORATION AND wilting of leaves have long been recognized as characteristic symptoms of chestnut blight, a serious disease of chestnut trees caused by the fungus *Endothia parasitica* (Murr.) A. and A. A general picture of the cause of the death of leaves on infected chestnut stems was first given by Murrill (1906), who ascribed death of the foliage following encircling of stems by *E. parasitica* to stoppage of food and water conduction under the lesions. This general relationship was accepted but not made more specific in subsequent publications on chestnut blight, owing to the fact that they were primarily concerned with phases of the disease other than its pathological physiology. Thus the extent to which conduction is interfered with prior to death of the foliage and the way in which the fungous infection acts to bring about interference and stoppage of the transpiration stream have not been described. The relative rapidity with which leaves are killed has indicated that death occurs as a result of some direct interference with the transpiration stream. The present paper describes changes in the sapwood which occur as a result of *E. parasitica* infection and the relationship of these changes to stoppage of water conduction and wilting of the foliage.

MATERIAL AND METHODS.—The study was carried out using chestnut sprouts growing in hardwood stands near New Haven, Connecticut, and potted chestnut seedlings. Both naturally infected trees and trees artificially inoculated with *E. parasitica* were studied.

A modification of the method of Farmer (1918) was employed for measuring the "water conductivity" of chestnut stems. The stem to be tested was severed at the base under water, or, if severed without immersion in water, the first cut was followed at once by a second cut made under water above the exposed end in order to obviate an entrance of air

into the sapwood. Three or four consecutive sections each 11 cm. long and spaced 1 cm. apart were cut from the severed stem while submerged under water. The series of stem sections thus obtained was numbered in order, 1, 2, 3, and 4, beginning with number 1 for the section taken nearest the base. These sections were carefully trimmed under water to 10 cm. in length and then put into a liter, wide-mouthed bottle containing sufficient water to cover them completely. An attachment was made to a water pump giving a suction equal to 74.3 cm. of mercury, and the sections were subjected to reduced pressure for 24 hours to remove free gases that otherwise would have caused a distinct variation in measurements. After this treatment, the sections were removed from the wide-mouthed bottle and attached, under water, to the rubber tubing of the apparatus used to determine water conductivity. This apparatus forced water through the section at a pressure equal to 1.5 cm. of mercury. Measurements of water conducted were made for three successive 15-minute periods, and an average of the three readings was taken as the water conductivity of the sections tested.

The removal of gases from stem sections by reduced pressure previous to tests for water conductivity probably creates a gas-liquid relationship not normally present in woody stems, so that tests made as in the present study hold only for totally water-filled wood, a condition which apparently is not normal in most trees (MacDougall, Overton, and Smith, 1929). The primary purpose of the tests in the present study being to detect mechanical blockage of conducting elements in the infected chestnut stems, it seemed advisable to remove the gases in order to obtain uniform tests, in spite of any changes from the normal that might ensue. Although the difference in water conductivity between individual sections from untreated stems was very irregular, the gradient actually obtained from a series of sections not exposed to reduced pressure showed the same general increase from the base upwards as that obtained with sections from which gases had been removed.

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Contribution from the Osborn Botanical Laboratory, Yale University. The writer wishes to thank Prof. Carl G. Deuber and Prof. J. S. Boyce for their guidance and helpful criticisms.

WATER CONDUCTIVITY OF UNINFECTED STEMS.—A study of the water conductivity of uninfected chestnut stems was made as a necessary forerunner to determination of changes brought about in stems by *E. parasitica* infections. This preliminary work was restricted to establishment of the gradient in water conductivity which could be expected when 3 to 4 consecutive sections, each 10 cm. in length, were taken from an uninfected chestnut stem and tested as previously described under "material and methods." The gradient thus obtained was taken to represent the relationship normally existing between the water conductivity of adjacent sections. By this method, a comparison could be made between the water conductivity of sections from the same stem as well as between gradients shown by series of consecutive sections from different stems, and from these comparisons a general quantitative evaluation of the decrease in conduction that occurred under *E. parasitica* lesions could be obtained.

Consecutive sections from uninfected chestnut stems representing a range of from 8.5 mm. to 12.0 mm. diameter inside the bark showed a slight increase in water conductivity from the base towards the apex (fig. 1A). Thus, section 1, taken from nearest the base, possessed the least total conductivity; section 2, a slightly greater conductivity than section 1; section 3, greater than section 2; and section 4, lying nearest the apex, possessed the greatest conductivity of the series. Such a gradient is of interest, as it is the reverse of that obtained from some other deciduous trees by Farmer (1918), Holmes (1919), and Rivett (1920), who found a decrease rather than an increase in total water conductivity from the base of shoots towards the apex. These investigators did record an increase in efficiency of the upper portion of the stem shoot, shown by water conductivity per sq. cm. of sapwood, but evidently the greater efficiency of upper portions of the stem was not sufficient to overcome the advantage of the larger cross-section of sapwood in the lower portions. A decrease in water conductivity proceeding from lower to upper stem sections was also obtained by Gardner (1925) working with pear trees.

CHANGE IN WATER CONDUCTIVITY OCCURRING IN INFECTED STEMS.—After the water conductivity of normal, uninfected chestnut stems had been studied, and the gradient which could be expected from successive stem sections had been established, similar tests were made with infected stems of the same diameters to determine whether an appreciable decrease was brought about under *E. parasitica* lesions. The infected stems were selected from sprouts and seedlings which had been inoculated with *E. parasitica* at a point midway between their bases and lowest branches so that the central section taken from such stems (section 2 or 3 in the conductivity series) would bear a lesion. From tests on those stems it was possible to compare the water conductivity gradient of a series of stem sections, one of which bore a fungous lesion, with the conductivity gradient of a

similar series from an uninfected stem, and thus to observe the displacement of the infected section from its normal place in the gradient. In the preceding

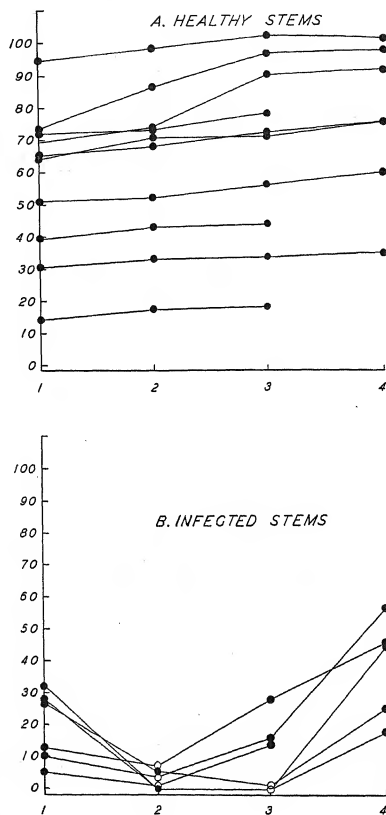


Fig. 1.—A. Water conductivity of 10 cm. sections taken from 10 healthy chestnut stems. Abscissa represents 4 consecutive sections numbered from base towards the apex; ordinate represents water conducted during 15 minutes in cubic cm.—B. Water conductivity of 10 cm. sections taken from 6 stems infected by *E. parasitica*. Abscissa represents 4 consecutive sections numbered from base towards apex; ordinate represents water conducted during 15 minutes in cubic cm. Black circles represent uninfected sections, white circles infected sections of each series. All trees possessed living foliage at time of measurement.

section (fig. 1) a normal conductivity series has been shown to possess a gradient which increases from the base towards the crown; thus, of the consecutive sections numbered 1, 2, 3, and 4, from the base to the apex, sections 2 or 3 had a total conductivity lying between those of sections 1 and 4. Decrease of the conductivity of infected sections 2 or 3 to a value less than that of section 1, or a total stoppage of conduction in 2 or 3, without any appreciable decrease in the conductivity of 1 and 4 was taken as a measure of the effect of the localized fungous infection on conduction.

According to the data collected during this study which are presented in figure 1B, there was a marked decrease in water conductivity brought about by *E. parasitica* infections. Furthermore, the effect was chiefly restricted to portions of the stem adjacent to fungous lesions, giving evidence of a localized effect on conduction. The effect was first exhibited by a displacement of infected sections, 2 or 3, from their

4 years old, which had been inoculated with *E. parasitica* were used for these observations. The trees were selected at various periods following encircling of their stems by bark lesions; the condition of their foliage was noted, and the path of the transpiration stream was determined in the following manner: The root system and lower portion of the stem of the tree to be observed were submerged in water and the stem was severed at the base. The severed end of the stem was placed in a dilute solution of light green dye (1:1,000), where it was allowed to remain for 24 hours in order to permit the dye to be carried by the transpiration stream through the stem to the crown. At the end of 24 hours the stem was sectioned, and the pathway of ascent of the dye as shown by a transverse view was diagrammed to obtain the conduction pattern. When the ascent of dye was irregular, or when no conduction was discernable, the apical end of the stem was attached to a water suction pump, and an effort was made to suck dye

TABLE 1. Conduction of dye through cankered portion of stems of infected chestnut trees. Trees were severed at the base and placed in dilute solutions of light green F.S. dye. The stems of all trees used were completely encircled by a lesion at approximately 2 feet from their bases.

Tree No.	Condition of leaves	Conduction of dye by sapwood under canker
1	Normal	Dye conducted by all vessels of outermost ring of sapwood
2	Normal	
3	Normal	
4	Normal	
5	Normal	
6	Normal	
7	Normal	Dye conducted by 50% of sapwood under canker
8	Pale-green, drooping	
9	Pale-green, not drooping	Dye conducted by 25% of sapwood under canker
10	Pale-green, drooping	
11	Pale-green and brown, drying	No conduction of dye by sapwood under canker
12	Dry	
13	Dry	

normal position in their respective conductivity series. A somewhat similar decrease was also shown by stem sections lying immediately below the infected section. As the disease progressed, those portions of the stem bearing lesions and the sections lying immediately below became totally blocked to conduction. Water could neither be forced through such diseased sections by pressure nor pulled through by suction. The stoppage of conduction remained localized even after the foliage had died, so that sections taken immediately above and sections taken more than 10 cm. below the infected sections were not found blocked to passage of water under pressure or suction.

For a check on measurements of water conductivity as an expression of reduction in conduction through infected chestnut stems, observations were made on conduction patterns of stems at various stages of infection. A series of young potted chestnut trees,

solution through the stem for comparison with the conduction pattern given by the transpiration stream. In addition to conduction patterns obtained from entire trees, dye was forced by pressure and by suction through the stem sections used in making measurements.

Results from observations on dye conduction by the transpiration stream indicated that a marked reduction in the effective cross-section of sapwood under the cankers took place before the foliage showed symptoms of dying (table 1). As long as the infection was restricted to the bark, dye was conducted by the outermost growth rings of sapwood in a manner similar to dye conduction in uninfected stems. When the leaves began to turn color or wilt, however, conduction was shifted to the inner rings, owing to infection and stoppage of conduction in the outer rings, with the result that 50 per cent or more of

the cross-sectioned area of sapwood became non-conducting. At a later stage, when the leaves turned brown and died, no dye conduction occurred through the infected portion of the stem, either under pull from the transpiring foliage or under suction of a water pump. A similar decrease in the dye conduction pattern was observed in stem sections used for conducting power tests—that is, infected sections whose conduction of water was abnormally low showed a corresponding reduction in the area of sapwood open to dye conduction. No differences in dye patterns of the latter were obtained when suction of a water pump (74.3 cm. Hg) was substituted for pressure of the conduction apparatus (1.5 cm. Hg).

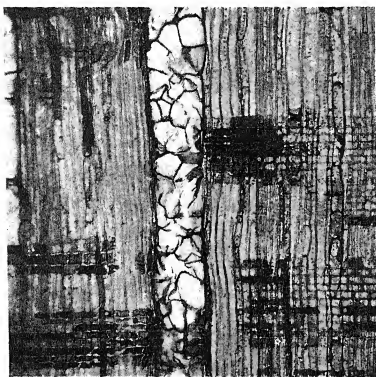


Fig. 2. Tyloses in a vessel in the youngest growth ring of sapwood of a diseased chestnut sprout (94X). The ray parenchyma are infected, and their tannin content is oxidized.

CAUSE OF CHANGE IN WATER CONDUCTIVITY IN INFECTED STEMS.—After a decrease in water conductivity under *E. parasitica* infection, which ultimately amounted to a complete stoppage of conduction, had been observed and measured, an attempt was made to determine the direct cause or causes of interference with conduction in infected chestnut stems.

A study of the progress of infection revealed that although hyphae of *E. parasitica* penetrated from bark lesions into the sapwood soon after infection reached the cambial region and were even to be found in the innermost layers of sapwood before the foliage died, mycelium was not present in conducting elements during early stages when conduction was first decreasing. This lack of mycelium in the conducting elements seemed to be due, in part at least, to early hyphal penetration taking place through wood and pith rays. Late stages in infection of ray parenchyma by the invading hyphae were easily followed as the tannin became oxidized to an amorphous, yellowish-brown

wound gum (fig. 2) which imparted a distinct yellowish-brown discoloration to the sapwood (Bramble, 1936). Invasion of conducting elements then took place from the rays, but, even in late stages, mycelium did not accumulate in the vessels (fig. 2).

Upon microscopic examination of the pathological anatomy of infected stems, an abnormal development of tyloses was observed to have occurred in reaction to infection. The tyloses appeared as bulbous outgrowths from parenchyma cells bordering vessels and extended out through pits into the lumina of the vessels. As the outgrowths enlarged, they filled the lumina, and their walls became angular and flattened as they pressed against each other and against the walls of the restricting vessels (fig. 2). Such outgrowths offered an effective block to passage of water through the sapwood and seemed to be the direct cause of the total stoppage of conduction peculiar to infected parts of chestnut stems. Formation of tyloses began in the outermost growth ring of sapwood and continued to form until the conducting elements of the entire sapwood of the infected portion of the stem were blocked.

Although the fundamental cause of tylosis formation in plants has not been satisfactorily settled, the appearance of such outgrowths in conducting elements seems to be a common reaction to fungous infection in trees capable of forming tyloses—i.e., with parenchyma cells adjacent to conducting elements. Bäsgen and Münch (1929), Wardlaw (1931), White (1910), and Schwarz (1922) have described tylosis formation as a reaction of sapwood to fungous infection. A similar disturbance to that in the cases mentioned above occurs in the sapwood of chestnut trees when infected by *E. parasitica*. Exactly what changes are brought about by the fungus which are responsible for tylosis formation have not been conclusively demonstrated, but it seems probable that some product was released by infected cells or by the fungus which brought about a growth stimulation of parenchyma cells bordering on vessels. Some support to such an explanation is given by Rumbold (1916), who found that slightly toxic or stimulating chemicals were able to cause a formation of tyloses in chestnut stems which resulted in plugging of the vessels. The development of tyloses in the outer, or youngest, rings of sapwood of the chestnut sprouts which occurred under *E. parasitica* lesions was restricted to those portions of the stem adjacent to the fungous infection and is believed to represent a host reaction to injury caused by the action of the fungus. The vessels of the outer rings of sapwood in young chestnut sprouts are normally free from tyloses, and even rings bordering on heartwood of young sprouts have been found open to passage of water; tyloses were found only in heartwood and in the transition zone between heartwood and sapwood.

SUMMARY

A study of the water conductivity of chestnut stems infected by *Endothia parasitica* has shown that complete stoppage of water conduction occurs prior to

the death of the foliage. This stoppage of conduction is found only in portions of the stems bearing *E. parasitica* bark lesions.

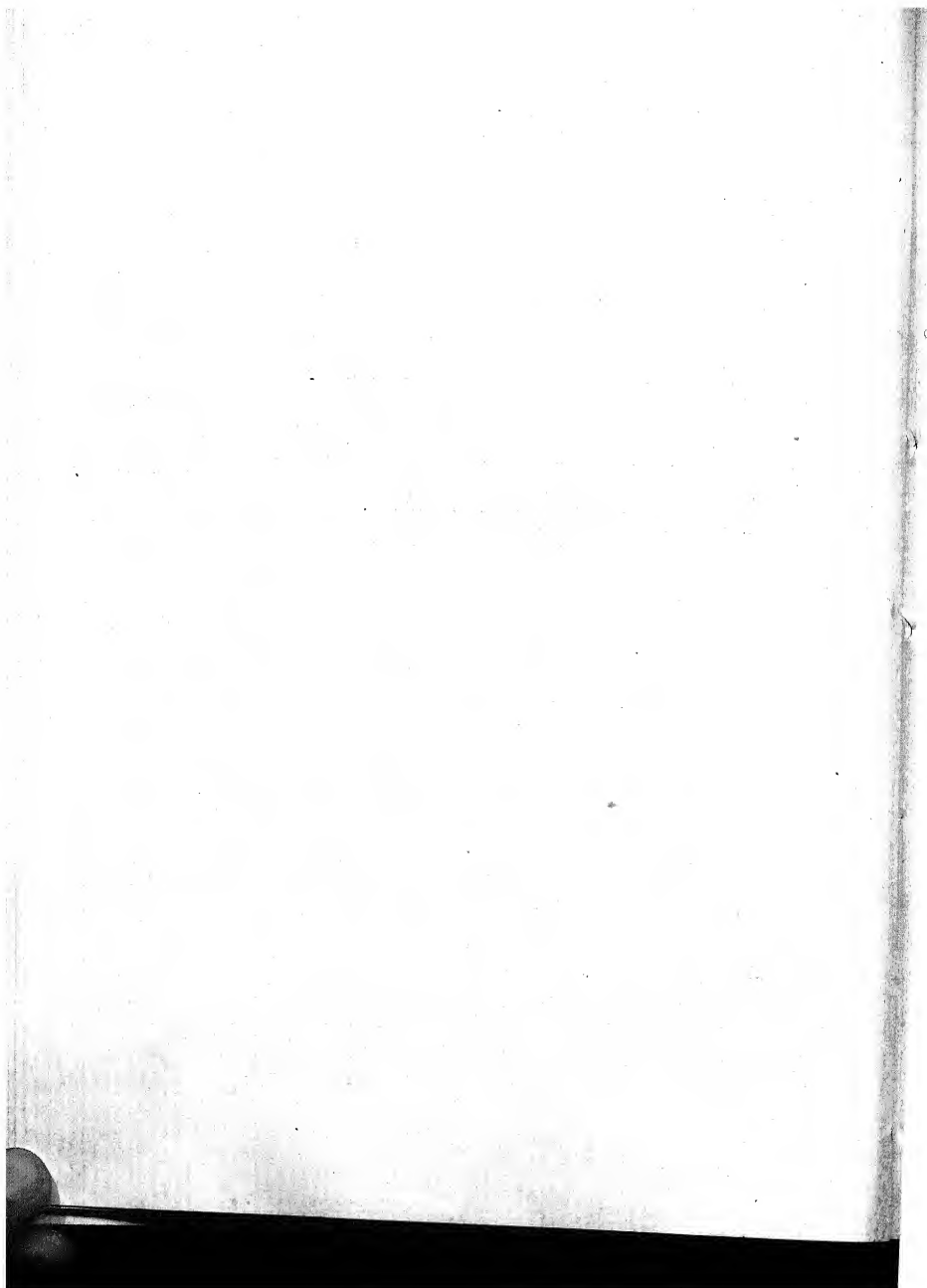
The most probable direct cause of stoppage of conduction under the fungous lesions is an abnormal formation of tyloses in the sapwood. The tyloses protrude into conducting elements and thus block the

passage of water through the stem. Such mechanical blocking of conducting elements by tyloses is held as the chief factor responsible for wilting and drying of leaves on infected chestnut stems.

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EXPERIMENTAL DATA FOR A REVISION OF THE GENUS LATHYRUS¹

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THIS investigation of the genus *Lathyrus* L. (Fam. Leguminosae; subfamily, Papilionatae) is an attempt to provide adequate biological data for an eventual revision of the genus. The data presented herein are concerned with chromosome number and, to a limited extent, chromosome morphology, morphology of the plant, epidermal cell-patterns of the leaf, interspecific crossing, and geographic distribution.

Lathyrus is an interesting genus for cyto-taxonomic study. It has a widespread distribution in north temperate regions and in South America. Some of its specific lines are clear cut and definite, while others are very vaguely defined. It has relatively large chromosomes, which, although in general similar to each other, do show some differential morphology. The outstanding cytological characteristic is the uniformity of chromosome number, forty-one species studied having $n = 7$ and one $n = 14$. Thus specific differentiation in the genus must have resulted almost entirely from structural changes in the chromosomes, changes in chromosome size, and genic changes.

Winge (1919), studying *L. latifolius* L. and *L. odoratus* L., reported the first chromosome numbers for the genus *Lathyrus*. Since that time chromosome number determinations have been reported by: Latter (1925, 1926, 1932), Punnett (1927), and Maeda (1928) on *L. odoratus* L.; Kawakami (1930) on *L. odoratus* L. and *L. japonicus* Willd. (*L. maritimus* Bigel.); Corti (1930, 1931a, 1931b) on *L. grandiflorus* Sibth. et Sm., *L. Aphaca* L., *L. articulatus* L. var. *clymenum*, and *L. niger* (L.) Bernh.; Melderis and Viksne (1931) on a group of nine species; Fisk (1931) on *L. tuberosus* L.; Simonet (1932) on a group of 23 species; Roy (1933, 1936) on *L. sativus*; and Fabergé (1935) on an aberrant tetraploid plant of *L. odoratus* L. Senn (1935-1936) reported eleven previously undetermined species. Senn (in press) listed the chromosome number reports for *Lathyrus* including determinations on 127 cultures of 35 species. The origin of each of these cultures was cited, and the relation of *Lathyrus* to the other genera of the tribe Viciae discussed.

The study of the genus *Lathyrus* was begun in 1931 at McMaster University, where plants were grown in the greenhouse and experimental plots and in the garden at Caledonia, Ontario. In 1934 the cultures were moved to The Blandy Experimental Farm, where they have since been maintained and expanded. The writer is grateful for helpful criticism and advice to Prof. Lulu O. Gaiser of McMaster University, under whose direction this study was initiated, and to Prof. Orland E. White of The Blandy Experimental Farm, University of Virginia, under whom it was continued.

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MATERIALS AND METHODS.—Seeds and plants of *Lathyrus* were obtained from botanic gardens, commercial establishments, and private individuals, to all of whom the writer is most grateful. *L. japonicus* Willd., *L. palustris* L., *L. ochroleucus* Hook., and *L. venosus* Muhl. were collected in their native habitats. The plants were grown in the experimental gardens of The Blandy Experimental Farm. As many cultures as possible of each species were grown in order to detect variations within the species.

Gametic chromosomes were studied in smears made with Belling's iron-aceto-carmine. In some instances the material was first fixed in Carnoy's fluid (3 parts chloroform, 2 parts absolute alcohol, 1 part glacial acetic acid). Somatic chromosomes were studied in root tip tissue, the roots being obtained from germinating seeds or from seedlings at the time of transplantation. Seeds of the species of the *Orobos* section proved particularly difficult to germinate. This difficulty was alleviated somewhat by refrigerating the seeds at 10°-15°C. for periods of two or three days to several weeks. It was found that alternate low and high temperatures sometimes hastened germination. The root tips were fixed either in Flemming's weak solution or Nawaschin's solution (10 parts of 1 per cent chromic acid, 4 parts commercial formalin, 1 part glacial acetic acid). Sections were made 10 μ in thickness and stained in Heidenhain's iron-alum haematoxylin or Newton's crystal violet. The drawings of chromosomes were made with a Zeiss microscope, 1/12 inch oil immersion objective N.A. 1.25, $\times 15$ ocular, using an Abbé camera lucida. All chromosome drawings are reproduced at a magnification of $\times 1960$.

Drawings of cells of the upper epidermis of leaves were made from thin strips of epidermis mounted in water. The drawings were made with the same microscope and camera lucida at a magnification of $\times 720$. They have been reduced in reproduction to $\times 120$. An effort was made to obtain typical epidermal cells for drawings. The epidermis lying over veins was always avoided, since it was characterized by long narrow cells regardless of the species and did not show any specific differentiation.

Typical calyces and styles were selected for drawing. One tube of a Bausch and Lomb binocular dissecting microscope, with the camera lucida, was used in making these drawings. The original magnification was $\times 11$, but the figures have been reduced to a magnification of $\times 4.1$.

Herbarium specimens of the material studied were preserved. Observations recorded on the morphology of the species were made on living plants in the field and in each instance represented a group of plants within a culture.

TAXONOMIC HISTORY.—Linnaeus (1753) described 21 species under the generic name *Lathyrus*. Under the generic name *Orobanch* he described 8 species which have since been included with the genus *Lathyrus*. Willdenow (1803) extended these descriptions to include 36 species under *Lathyrus* and 13 under *Orobanch*.

The first and only comprehensive treatment of the genus *Lathyrus* is that of Alefeld (1861). He separated 61 species now known as *Lathyrus* into 8 distinct genera and devised a classification for the tribe Viciaceae, which he considered as a family. Alefeld's genera, *Clymenum*, *Graphiosa*, *Aphaca*, *Orobanch*, *Lastila*, *Navidura*, *Cicerula*, and *Lathyrus* are included in the genus *Lathyrus* as it is understood today.

Bentham and Hooker (1862-1867) established the present concept of the genus, uniting Alefeld's 8 genera into the one genus *Lathyrus*. The genus *Orobanch*, which sometimes is still regarded as a distinct genus, was treated by Bentham and Hooker as a section of *Lathyrus*. Taubert (1894) arranged the species of the genus in two sections, *Archilathyrus* and *Orobanch*, dividing the first section into six subsections. The major division into sections was based on the presence of tendrils on the leaves of the species *Archilathyrus* and the absence of tendrils in *Orobanch*.

In America, White (1894) published a revision of the *Lathyrus* of North and Central America. Thirty-three species were listed, including some new species. A key to these species was given, but no attempt made to orient the American species within the whole genus. The west coast species of *Lathyrus* have been the most difficult American species to evaluate taxonomically. Piper (1918) discussed several western species and established specific lines for them. Bradshaw (1925) described 24 Pacific coast species and prepared keys to the species and subspecies. His revision does not discuss the American species in relation to the species of the rest of the world, so the relationships of the endemic American species of *Lathyrus* are still largely unknown.

In South America the only extensive treatment of the genus has been Burkart's (1935) revision of the species of the Argentine. He discusses and describes 26 native and introduced species. Again local species have not been related to the whole genus, since Burkart did not study the twisting of the style.

In Europe, Ascherson and Graebner (1906-1910) described 31 species of the genus and proposed another classification of these species. This classification of the genus arranged the species in seven sections grouped in two main divisions. The outstanding character separating these divisions was the twisting on its axis of the pistil and sometimes also the stamens and keel in the sections *Cicerula* and *Eulathyrus*. Since most of the species studied in this investigation were European species and since some, at least, of the American species fit easily into this arrangement, it has been used throughout. When a complete revision of the genus is made, this classification may

prove inadequate, but at present it is the best which has been proposed.

GEOGRAPHIC DISTRIBUTION.—Data on the distribution of the genus *Lathyrus* have been assembled from two chief sources. It is recognized that ultimately the accurate method of determining the distribution of a genus or species is the examination of large numbers of herbarium specimens from as many localities as possible. Failing this, the data from Index Kewensis (Hooker, Jackson, et al., 1895—) and from authoritative regional floras have been compiled and classified.

Index Kewensis lists 281 "good" species of *Lathyrus*. Careful comparative morphological studies will probably reduce a number of these species to the status of synonyms. Since no modern monograph of the genus is available, these data are at present the only working basis for a comprehensive study of the genus.

The distribution of the genus may be presented by considering the number of species in the following six areas: Mediterranean region (including southern Europe, North Africa, and Asia Minor), 76 species; Europe, Africa, and Asia (outside the Mediterranean region), 43, 11, and 47 species, respectively; North and Central America, 58 species; and South America, 46 species.

From the above data it is seen that the tropics, except for high mountains, Australia and New Zealand, and temperate South Africa are all without representatives of the genus. In contrast, there are many species scattered throughout temperate Europe and Asia, with by far the greatest number localized in the Mediterranean region. In North America there are a few species spread across Canada and eastern United States, with a large number of species in western United States. There are a few species which follow the Rocky Mountain cordillera through Mexico and Central America into South America. Here there are a large number of species in the Argentine and Chile with a few extending to the southernmost tip of the continent. Burkart (1935) has carefully analyzed the distribution of the species of the Argentine. There are, according to him, 25 species native to South America, and of these, 21 are native to the Argentine. Burkart has prepared maps of the distribution of the Argentine species based on herbarium specimens. These indicate that the centers of distribution of the Argentine species are located in the regions of the La Plata river and the coast of Argentina, Uruguay, Rio Grande do Sul of Brazil, and the forests of Patagonia.

Only two species are found in both the old and new worlds. *L. palustris* L. is found in marshlands throughout the north temperate zone, and *L. japonicus* Willd. (*L. maritimus* Bigel.) is found along the coasts of Europe, Asia, and North America. It has also been reported from the coast of Chile in South America, but, according to Burkart (1935), it was introduced there. Since only the European species have been adequately arranged in natural sections,

the world distribution of the sections cannot be fully discussed. However, the two species mentioned above both belong to the *Orobastrum* section, indicating that it is present in America as well as Europe. It was found in the present study that *L. nigricaudis* A. Burk., a South American species, is also a member of this section. These species may prove to be the link between the European species and those in America. It is suggested from the number of species present and the fact that all sections of the genus are represented that the Mediterranean region is the primary center of distribution of the genus. Western United States and the Chile-Argentina region of South America probably represent secondary centers of distribution, for in both of these regions relatively large numbers of species are found. Both of them also have several highly polymorphic species. The distribution of each species will be cited in detail under the discussion of the species.

EXPERIMENTS IN INTERSPECIFIC HYBRIDIZATION.—Interspecific compatibilities provide a valuable clue to the evolutionary relationships between species. In addition to the theoretical importance of species-crossing in *Lathyrus*, certain species-hybrids would probably be of considerable horticultural value. Taylor (1916) claimed to have successfully made the cross *L. odoratus* L. × *L. pratensis* L. and the reciprocal of this cross. Barker (1916) reported that he had crossed *L. odoratus* L. with *L. hirsutus* L. Melderis and Viksne (1931) reported unsuccessful attempts to make crosses between *L. articulatus* L., *magellanicus* L., *odoratus* L., *pratensis* L., *tingitanus* L., and *vernus* Bernh. and *Pisum sativum* L. The taxonomic literature contains some references to interspecific hybrids, but since the evidence is not experimental it cannot be considered as adequate. Zvolanek has traced the history of his winter-flowering sweet peas to crosses he claims to have made between *L. odoratus* L. and the garden pea (*Pisum sativum* L.) and a common vetch (*Vicia* sp.). The historical evidence indicates that interspecific crosses in *Lathyrus* have been successful only very rarely, if at all.

During 1932 and 1933 unsuccessful attempts to cross *L. latifolius* L., *L. odoratus* L., and *L. sativus* were made in the greenhouse and garden at McMaster University. An extended series of interspecific crosses was undertaken at The Blandy Experimental Farm in 1935 and 1936. Seventeen species of *Lathyrus* and *Pisum sativum* L. were used in 458 attempts at interspecific and intergeneric crosses. The crosses attempted are listed below, the pistillate parent always being stated first.

- L. annuus* L. with *L. Cicera* L. and *L. sativus* L.;
L. Aphaca L. with *L. sativus* L., *L. numidicus* Batt., *L. latifolius* L., *L. Cicera* L., *L. Clymenum* L., and *L. articulatus* L.;
L. articulatus L. with *L. tingitanus* L., *L. sativus* L., *L. Cicera* L., *L. Clymenum* L., *L. Nissolia* L., *L. numidicus* Batt., and *L. Ochrus* DC.;
L. Cicera L. with *L. sativus* L., *L. Clymenum* L., and *Pisum sativum* L.;

L. Clymenum L. with *L. sativus* L., *L. articulatus* L., *L. latifolius* L., *L. odoratus* L., *L. Ochrus* DC., and *Pisum sativum* L.;

L. heterophyllus L. with *L. latifolius* L. and *L. sylvestris* L.;

L. latifolius L. with *L. tingitanus* L., *L. odoratus* L., *L. sativus* L., *L. Cicera* L., *L. heterophyllus* L., *L. Clymenum* L., *L. sylvestris* L., and *Pisum sativum* L.;

L. numidicus Batt. with *L. latifolius* L. and *L. niger* (L.) Bernh.;

L. Ochrus DC. with *L. sativus* L., *L. articulatus* L., *L. Clymenum* L., *L. Cicera* L., *L. Aphaca* L., *L. latifolius* L., *L. tingitanus* L., and *Pisum sativum* L.;

L. odoratus L. with *L. Clymenum* L., *L. latifolius* L., *L. tingitanus* L., *L. tuberosus* L., and *L. sativus* L.;

L. sativus L. with *L. Aphaca* L., *L. articulatus* L., *L. Cicera* L., *L. Clymenum* L., *L. latifolius* L., *L. niger* (L.) Bernh., *L. numidicus* Batt., *L. Ochrus* DC., *L. tingitanus* L., and *Pisum sativum* L.;

L. sylvestris L. with *L. heterophyllus* L. and *L. latifolius* L.;

L. tingitanus L. with *L. sativus* L., *L. latifolius* L., *L. Cicera* L., *L. numidicus* Batt., *L. odoratus* L., *L. articulatus* L., *L. tuberosus* L., and *L. sylvestris* L.;

L. tuberosus L. with *L. latifolius* L.;

L. venosus Muhl. with *L. niger* (L.) Bernh., *L. tuberosus* L., and *Pisum sativum* L.;

L. niger (L.) Bernh. with *L. venosus* Muhl., and *L. sativus* L.;

Pisum sativum L. with *L. latifolius* L., *L. numidicus* Batt., *L. niger* (L.) Bernh., *L. venosus* Muhl., and *L. sativus* L.

In most instances the pollinated flowers merely dropped off a few days after pollination. In 10 crosses pods were formed either without seeds or containing badly shrunken seeds. Two of these were crosses between the closely related *L. articulatus* L. and *L. Clymenum* L. and two between the related *L. Ochrus* DC. and *L. Clymenum* L. One of these crosses was that between *L. latifolius* L. and *L. tingitanus* L., both species of the *Eulathyrus* section. In the remainder of the crosses which formed immature pods, the species were not closely related taxonomically.

In four instances seeds were set. These crosses were: *L. Aphaca* L. × *L. numidicus* Batt., *L. Cicera* L. × *L. sativus* L., *L. Ochrus* DC. × *L. Cicera* L., and *L. niger* (L.) Bernh. × *L. venosus* Muhl. Plants grown from the first three of these crosses proved to be entirely like the maternal parent in appearance, so that it is probable some contamination with maternal pollen had occurred. The seeds from the last cross failed to germinate.

The reasons for this interspecific incompatibility are not at all evident. Practically all the plants used had the same chromosome number, so purely numerical difficulties are not involved. The stylar length in many of the crosses was nearly, if not quite, equal, so differences in pollen tube length or heterostyly should not be involved. Structural chromosomal dif-

ferences may be so great as to prevent proper pairing, or the incompatibility may result from chemical differences in the stigmatic and stylar tissue of the various species, so that pollen tube growth is inhibited. The whole problem demands a careful experimental study.

CYTOTOLOGICAL AND MORPHOLOGICAL OBSERVATIONS.—The species discussed below have been carefully studied in experimental plots, and chromosome number determinations made. Detailed measurements of the various organs of the plant will not be cited in this paper. Space permits figuring only the styles, calyces, and epidermal cells of one representative species from each section of the genus.

Section *Cicerula* Moench.

The flowers of this section of the genus have the pistil, and sometimes also the stamens and keel, twisted so that the broad surfaces face right and left. The plants are weak to strong climbers, with leaves consisting usually of one pair of leaflets and a curled tendril. The flower stalk usually is one-flowered and shorter than the subtending leaf. The pod is linear to ovate and contains 3-5 seeds. All the species studied are annuals except *L. hirsutus* L., which is biennial under some conditions.

The pistils of this section (fig. 1) are in general short (from 0.3-0.6 cms. long) and somewhat broadened toward the stigmatic region. They differ from those of the section *Eulathyrus* Ser. (fig. 14) chiefly in that the latter are much larger. The calyces of the species of the *Cicerula* section (fig. 2) are similar, differing from each other chiefly in size. They are all characterized by the calyx tube tapering gradually to the peduncle in contrast to the calyces of *Eulathyrus* (fig. 6), where the tube is truncate at the base.

The epidermal cell patterns of the species studied are similar (fig. 51), the cells being usually 2-4 times as long as broad, with moderately undulated walls. Chromosome numbers were determined for five species, and all were found to be $n=7$, $2n=14$.

L. annuus L. $n=7$, $2n=14$.—This species grows in dry places throughout the Mediterranean region of Europe, Asia, and Africa. The six cultures studied proved to be fairly uniform. Meiosis was regular (fig. 15). The somatic chromosome complement (fig. 34) consisted of 2 pairs of long chromosomes with median constrictions, 2 pairs of long chromosomes with submedian constrictions, 2 pairs of medium length, median constricted chromosomes, and 1 pair of medium length and submedian chromosomes.

L. Cicera L. $n=7$, $2n=14$ (fig. 16, 35).—This species occurs throughout the whole Mediterranean region, and also in the Canary Islands, Syria, Persia, and Transcaucasia. Thirteen cultures were studied, and all proved to be fairly uniform. Meiosis was regular.

L. hirsutus L. $2n=14$.—This species occurs throughout the whole Mediterranean region and has been introduced into northern and central Europe

and east to Crimea, Transcaucasia, and Mesopotamia. Two cultures were grown, and the $2n$ chromosome number was determined as 14 (fig. 36).

L. numidicus Batt. $n=7$, $2n=14$.—This is a small annual species from Algeria which blooms very early and completes its life cycle in about six weeks. The plant is a semi-climber, the leaves having two leaflets and ending in a curled tendril. There is usually one flower per peduncle; the peduncle is shorter than the subtending leaf. The pistil is twisted on its axis. The pod is linear-cylindrical and usually contains three mature seeds. This species has not been previously placed in any of the sections of the genus, but on the basis of the characters just enumerated, it clearly belongs in the *Cicerula* section. One culture showed some irregularity in meiosis, but the other was consistently regular, and the chromosome number was determined as $n=7$, $2n=14$ (fig. 17, 33).

L. sativus L. $n=7$, $2n=14$ (fig. 1, 2, 18, 37, 51).—The original distribution of this species is difficult to determine since it has been widely cultivated in some parts of Europe and Asia for a long period of time. Its probable original distribution is the Mediterranean region, southwestern Asia, and northern India.

Fifty-seven cultures of this species were grown, many of which were provided by the courtesy of the Institute of Plant Industry of the U. S. S. R. These seeds came from widely separated regions. Since this species exhibits a considerable amount of variation, especially in flower color and seed size and color, morphological data were recorded on 45 cultures. Successful crosses were made between several of these cultures. Howard and Abdur Rahman Khan (1928) have assembled, grown, and analyzed the chief horticultural forms of the species in existence in India. They found 56 "unit species" which were grouped into three varieties on the basis of flower color. These were: var. *albus* with white flowers, one type; var. *roseus* with pink or red flowers, ten types; var. *cyaneus* with blue flowers, 45 types. There was considerable variation in the types of markings on the pods and great variation in the color and pattern of the seed coats. In the present study it was possible to distinguish seven chief types as regards flower color. These types are relatively constant when grown from year to year, and most of the cultures studied were found to contain only one color type.

Data were also taken on the variation in seed coat color in the cultures studied. In general, the seed coat pattern consists of a ground color with either no, one, or two superimposed layers of color. The amount and arrangement of the pigment in these layers varies considerably. There is also a variation in the color and arrangement of the hilum band and in the size of the seeds. For example, one culture had a seed size of 0.5-0.6 cms. diameter, and another culture had a size of 1.4-1.7 cms. diameter.

The cells of the upper leaf epidermis in *L. sativus* L. (fig. 51) are characteristically very long in relation to their width, and the cell walls are moderately

undulated. The gametic chromosome number was determined as $n=7$ in 30 cultures (fig. 18). All the cultures examined showed regular meiosis and good pollen grains. It is difficult to obtain preparations of the somatic chromosomes of this species which are clear enough to permit the chromosome morphology to be accurately determined. With the fixatives used, the chromosomes do not show sharp constrictions as many other species do. The somatic chromosomes were counted as $2n=14$ in 7 cultures (fig. 37). This species is a good example of the extreme chromosomal uniformity in the genus *Lathyrus*. Here the chromosome number and chromosome size is uniform over a group of varieties originating in very widely distributed geographical regions and exhibiting a relatively great degree of variation within the species.

This species is used rather extensively as a field crop, especially in India, and some varieties are used in the horticultural trade in Europe and America, especially under the name *Lathyrus azureus*.

Section *Eulathyrus* Ser.

This section is differentiated from the preceding section by the flower stalks bearing many flowers and exceeding the subtending leaf in length and by the long-linear, many-seeded pods. The species of the section have long styles (0.6-1.4 cms. long) (fig. 14) and large calyces (fig. 6) which are truncate at the base. The epidermal cells are in general somewhat broader than those of the preceding section, and there are fewer undulations in the walls. The eight species studied cytologically were all normal diploids, $n=7$, $2n=14$. Fabergé (1935) found a tetraploid form, $2n=28$, in a culture of *L. odoratus* L.

L. cirrhosus Ser. $n=7$.—This perennial species is found only in the Pyrenees Mountains. The epidermal cell pattern is very distinctive. The cells are rather irregular in shape, many of them being as broad as long. The walls have few or no undulations. The upper surface of the leaf is pubescent, two types of trichomes being found, a long narrow unicellular type, and a short, broad multicellular type. One culture was grown and the chromosome number determined as $n=7$ (fig. 23).

L. heterophyllus L. $2n=14$.—This species is limited to Europe and probably is not found in Portugal or Spain. Gams (1926) includes it along with two other perennials, *L. sylvestris* L. and *L. latifolius* L., under a collective species *L. silvester* L. *sensu lato*. This arrangement has not been followed here because the three species have not been adequately studied as yet, particularly from the standpoints of herbarium material and geographic distribution. The morphological data recorded indicate that these species are very closely related.

Two cultures of *L. heterophyllus* were studied. In one the $2n$ chromosome number was determined as 14 (fig. 40). The somatic complement consisted of three pairs of long, slightly submedian chromosomes, one pair of long submedian chromosomes, one pair of long submedian chromosomes with a secondary con-

striction in the shorter arm, one pair of medium length, slightly submedian chromosomes, and one pair of short median chromosomes. The other culture was exceedingly vigorous vegetatively but proved to be irregular cytologically. Repeated examination showed that both of the meiotic divisions were irregular. Almost all the tetrads and pollen grains were abnormal, tetrads with 5 and 6 nuclei occurring. No chromosome count could be made in the pollen mother cells. Very little seed was set throughout the blooming period, but toward the end of the period a few partially filled pods were obtained.

L. grandiflorus Sibth. and Sm. $2n=14$.—This species occurs in Italy and Greece. One culture was grown and the $2n$ chromosome number determined as 14.

L. latifolius L. $n=7$, $2n=14$ (fig. 6, 14, 19, 38, 52).—This familiar perennial species is distributed through southern Europe from France to central and southern Russia. It is widely cultivated, and many escapes make the determination of its exact distribution difficult. In an effort to study the variation in

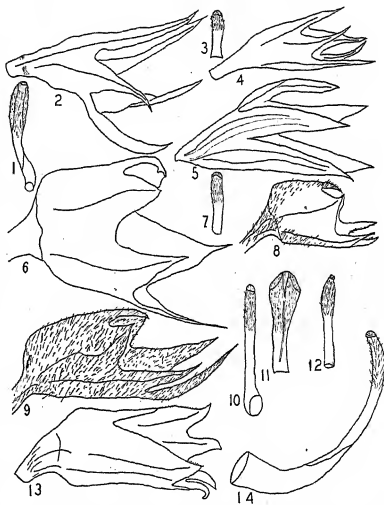


Fig. 1-14. Pistils and calyces of *Lathyrus*; camera lucida drawings from living material, magnification $\times 4.1$. Section *Cicerula* Moench.—Fig. 1, 2. *L. sativus* L. Section *Eulathyrus* Ser.—Fig. 6, 14. *L. latifolius* L. Section *Aphaca* Tourn.—Fig. 5, 7. *L. aphaca* L. Section *Nissolia* Tourn.—Fig. 3, 4. *L. Nissolia* L. Section *Clymenum* DC.—Fig. 11, 13. *L. Clymenum* L. Section *Orobastrum* Gren. and Godr.—Fig. 9, 10. *L. venosus* Muhl. Section *Orobis* (Tourn.) Taub.—Fig. 8, 12. *L. niger* (L.) Bernh.

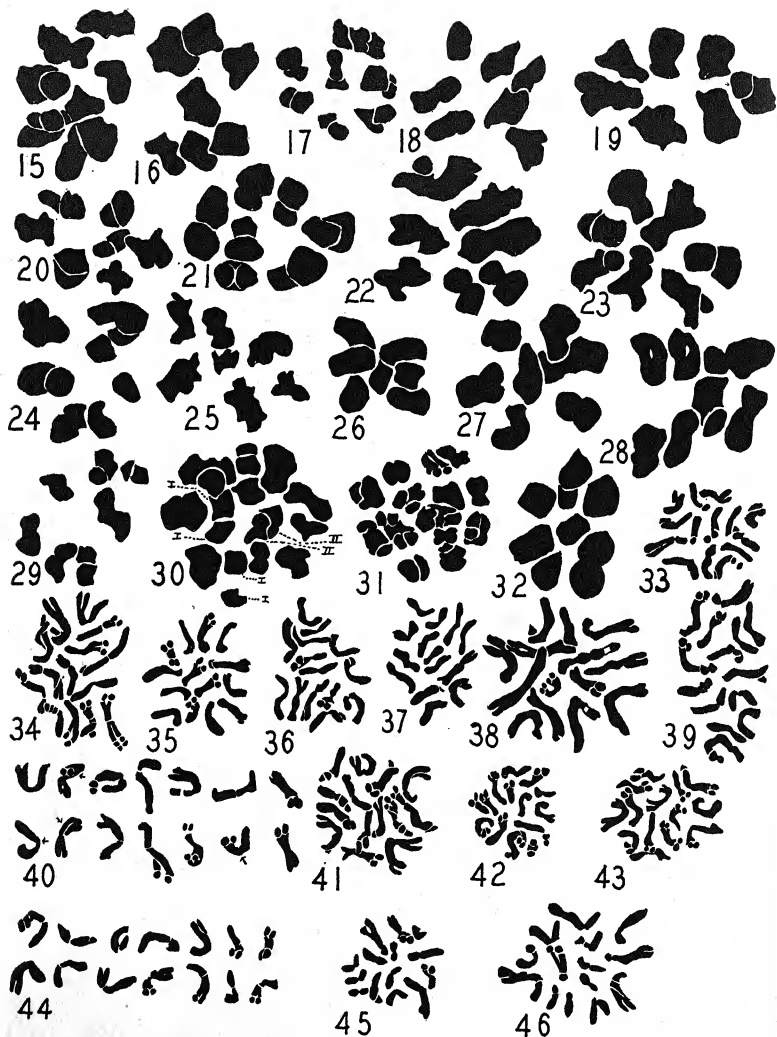


Fig. 15-46. Gametic and somatic chromosomes of *Lathyrus*; camera lucida drawings of I M in pollen mother cells and of metaphases in root tip cells, magnification $\times 1960$. Section *Cicerula* Moench.—Fig. 15. *L. annuus* L. $n=7$.—Fig. 16. *L. Cicera* L. $n=7$.—Fig. 17. *L. numidicus* Batt. $n=7$.—Fig. 18. *L. sativus* L. $n=7$. Section *Eulathyrus* Ser.—Fig. 19. *L. latifolius* L. $n=7$.—Fig. 20. *L. odoratus* L. $n=7$.—Fig. 21. *L. tingianus* L. $n=7$.—Fig. 22. *L. sylvestris* L. $n=7$.—Fig. 23. *L. cirrhosus* Ser. $n=7$. Section *Clymenum* DC.—Fig. 24. *L. Clymenum* L. $n=7$.—

this species, about 39 original cultures were grown. A number of progenies of selected plants in these cultures were also grown. Despite the extensive list of varietal forms discussed by Ascherson and Graebner (1906-1910), there are very few forms in cultivation, and the forms obtained from botanical gardens are limited almost entirely to these. Culture 13.1 from the University of Montpellier Botanic Garden was received as *L. ensifolius* Bad. but is here considered as a variety of *L. latifolius* L. Similarly, culture 50.1 from the University of Vienna Botanic Garden was received as *L. purpureus* Gilib. but is here considered to consist of varietal forms of *L. latifolius* L.

The chief variations to be observed have been three flower colors—white, pink, and red or deep pink—and a few variations in leaf size and shape. Culture 13.1, the form known as variety or species *L. ensifolius* Bad., is very distinctive since it has exceedingly narrow linear leaflets and floral characters like typical *L. latifolius* L. The gametic chromosome number $n=7$ was determined in 25 cultures (fig. 19) and the somatic number $2n=14$ (fig. 38) in 8 cultures.

L. sylvestris L. $n=7$, $2n=14$.—The range of this vigorous perennial species includes almost all of Europe, north to Scandinavia and east to the Caucasus. Fifteen cultures were grown. The epidermal cell patterns of broad and narrow-leaved forms are similar and very much like those of *L. latifolius* L. Gametic chromosome numbers ($n=7$, fig. 22) were determined in eight cultures. The somatic chromosome complement ($2n=14$) consists of 1 pair of long, median constricted chromosomes, 1 pair of long, slightly submedian chromosomes, 1 pair of long, submedian chromosomes with a secondary constriction in the shorter arm, 2 pairs of medium length median chromosomes, and 2 pairs of short median chromosomes.

L. odoratus L. $n=7$, $2n=14$.—The natural distribution of this familiar annual species is limited to Sicily. Fifteen cultures were grown and the gametic chromosome number determined as $n=7$ (fig. 20) in three of them, of which one was a dwarf form. The somatic chromosome complement (fig. 44) consists of 2 pairs of long chromosomes with slightly submedian constrictions, 1 pair of long submedian chromosomes, 2 pairs of medium length, submedian chromosomes, and 2 pairs of short median chromosomes.

L. tingitanus L. $n=7$, $2n=14$.—This annual species occurs in Spain and northwestern Africa around the Mediterranean. Little variation was observed in the nine cultures grown. Gametic chromo-

somes counts were made on four cultures ($n=7$, fig. 21) and somatic counts on three cultures ($2n=14$, fig. 41). The somatic chromosome complement consists of 1 pair of long median chromosomes, 1 pair of long submedian chromosomes, 3 pairs of medium length median chromosomes, and 1 pair of medium length, slightly submedian chromosomes. This species was significant because it had no representative of the short group of chromosomes.

L. tuberosus L. $n=7$, $2n=14$.—This is a perennial species with tuberous rootstocks which is distributed over almost all Europe, northern Africa, and western Asia. Of all the species studied, it is the only one besides *L. odoratus* L. that has fragrant flowers. Four cultures were grown and gametic chromosome numbers determined as $n=7$ in two of them. The somatic chromosome complement (fig. 39) is made up of 1 pair of long submedian chromosomes with a secondary constriction in the longer arm, 4 pairs of medium submedian chromosomes, and 2 pairs of medium length, medianly constricted chromosomes.

Section *Aphaca* Tourn.

In this and the following four sections the pistil is not twisted on its axis (fig. 7, 3, 11, 10, 12). The *Aphaca* section is characterized by leaf-like stipules and the reduction of the leaf so that it consists of only a curled tendril. The flowers are yellow and the standard has no tubercle at its base. This small section consists of only one species or of a small group of closely related species.

L. Aphaca Tourn. $n=7$, $2n=14$ (fig. 5, 7, 42).—This small annual is found throughout Europe, western Asia, and northern Africa. Seven cultures were grown and the gametic chromosome number determined as $n=7$. The somatic chromosome complement (fig. 42) consists of 2 pairs of long submedian chromosomes, 2 pairs of medium length, median chromosomes, 2 pairs of medium length, submedian chromosomes, and 1 pair of short median chromosomes.

Section *Nissolia* Tourn.

This section has small stipules and leaves which consist only of petioles broadened into phyllodes which end in short bristles instead of tendrils. The flowers are purple in color.

L. Nissolia L. $2n=14$ (fig. 3, 4, 43, 53).—This is also a small annual which is distributed throughout the whole Mediterranean region of southern Europe, northern Africa, and western Asia. Three cultures

Fig. 25. *L. Ochrus* DC. $n=7$. Section *Orobastrum* Gren. and Godr.—Fig. 26. *L. palustris* L. $n=7$.—Fig. 27. *L. setifolius* L. $n=7$.—Fig. 28. *L. nigralvovis* A. Burkart $n=7$.—Fig. 29. *L. sphaericus* Retz. $n=7$.—Fig. 30. *L. venosus* Muhl., Edmonton, Alberta. $n=14$, 12 bivalents and 4 univalents.—Fig. 31. *L. venosus* Muhl., Fort Valley, Virginia. $n=14$, 14 bivalents. Section *Orobis* (Tourn.) Taubert.—Fig. 32. *L. niger* (L.) Bernh. $n=7$. Somatic chromosomes. Section *Cicerula* Moench.—Fig. 33. *L. numidicus* Batt. $2n=14$.—Fig. 34. *L. annuus* L. $2n=14$.—Fig. 35. *L. Cicera* L. $2n=14$.—Fig. 36. *L. hirsutus* L. $2n=14$.—Fig. 37. *L. sativus* L. $2n=14$. Section *Eulathyrus* Ser.—Fig. 38. *L. latifolius* L. $2n=14$.—Fig. 39. *L. tuberosus* L. $2n=14$.—Fig. 40. *L. heterophyllus* L. $2n=14$ (arrows indicate spindle fibre attachment region).—Fig. 41. *L. tingitanus* $2n=14$.—Fig. 44. *L. odoratus* L. $2n=14$. Section *Aphaca* Tourn.—Fig. 42. *L. Aphaca* L. $2n=14$. Section *Nissolia* Tourn.—Fig. 43. *L. Nissolia* L. $2n=14$. Section *Clymenum* DC.—Fig. 45. *L. articulatus* L. $2n=14$.—Fig. 46. *L. Clymenum* L. $2n=14$.

were studied and the somatic chromosome complement (fig. 43) found to consist of 3 pairs of long submedian chromosomes, 2 pairs of medium length, median chromosomes, and 2 pairs of medium length, submedian chromosomes.

Section *Clymenum* DC.

This section differs from the last two in that the standard of the flower has two tubercles at its base. The petioles are broadened to a phyllode. The lower leaves on the plant have no leaflets, but the upper leaves may have a variable number of leaflets. Three closely related annual species in this section were studied. Their morphology, shape and size of the calyces and styles, epidermal cell patterns, and chromosome morphology indicate that they had a common origin.

L. Clymenum L. $n=7$, $2n=14$ (fig. 11, 13, 24, 46, 54).—This species occurs in the Mediterranean region and the Madeira Islands. It and the following species are sometimes regarded as varietal forms of one species (Coste, 1901; Gams, 1926). *L. Clymenum* L. may be differentiated from *L. articulatus* L. by the presence of a recurved projection from the stigmatic region of the pistil of the former (fig. 11) and by the wing petals of the flower being white in *L. articulatus* L. and mauve in *L. Clymenum* L.

The epidermal cell patterns of these species and of *L. Ochrus* DC. are similar, but that of *L. Clymenum* L. (fig. 54) characteristically shows smooth walls, while the walls of *L. articulatus* L. are somewhat undulated. Eleven cultures of *L. Clymenum* L. were grown and gametic chromosomes counted as $n=7$ (fig. 24) in two of these. In two other cultures the somatic number was determined as $2n=14$ (fig. 46).

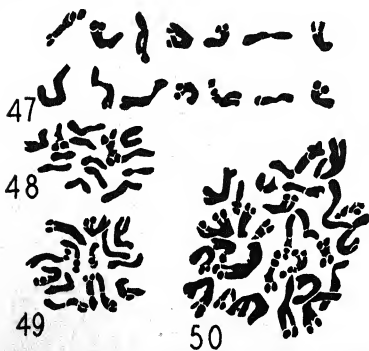


Fig. 47-50. Somatic chromosomes of *Lathyrus*; camera lucida drawings of metaphases in root tip cells, magnification $\times 1900$. Section *Orobastrum* Gren. and Godr.—Fig. 47. *L. japonicus* Willd. $2n=14$.—Fig. 48. *L. inconspicuus* L. $2n=14$.—Fig. 50. *L. venosus* Muhl. $2n=28$. Section ?.—Fig. 49. *L. crassipes* Gillies. $2n=14$.

The complement contained two pairs of short chromosomes, and this was found to be true of *L. articulatus* L. (fig. 45) and *L. Ochrus* DC. (fig. 25) also.

L. articulatus L. $2n=14$.—This species is limited to the western Mediterranean region. Five cultures were grown and the somatic number determined as $2n=14$ (fig. 45) in three of them.

L. Ochrus DC. $n=7$, $2n=14$.—This is a yellow flowered species which is found throughout the Mediterranean region. Ten cultures were grown and gametic chromosome counts ($n=7$, fig. 25) made in two of them and somatic counts ($2n=14$) in one.

Section *Orobastrum* Gren. and Godr.

The species of this section have pinnate leaves ending in divided tendrils. They are usually weak climbers. The pistils (fig. 10), with the exception of that of *L. nigralvis* A. Burkart, are narrow, broadening but little toward the stigma. They vary considerably in length from that of *L. sphaericus* Retz. (0.2 cms. long) to that of *L. venosus* Muhl. (0.6 cms. long). All the species of northeastern United States and eastern Canada and at least one South American species belong to this section of the genus.

L. japonicus Willd. (*L. maritimus* Bigel.). $2n=14$.—This perennial species occurs along the sea coasts of the arctic and north temperate regions of Asia, Europe, and North America at least as far south as New York and also along the shores of the Great Lakes and the St. Lawrence river. It is commonly reported as occurring in South America, but Burkart (1935) considers it to have been introduced there. Fernald (1932) discussed the taxonomy of the species, changing the name from *L. maritimus* Bigel. to *L. japonicus* Willd. and describing four chief varieties. Regel (1935) retained the name *L. maritimus* (L.) Bigel. and described two major subspecies. This species was collected at Sauble Beach, Lake Huron, Gray County, Ontario. A culture from Midland, Ontario, had a somatic chromosome complement (fig. 47) composed of 1 pair of long median chromosomes, 2 pairs of long submedian chromosomes, 3 pairs of medium length, median chromosomes, and 1 pair of short median chromosomes.

L. palustris L. $n=7$.—This species is a perennial which is circumpolar in distribution, being found throughout the north temperate zone. It has not been cultivated successfully in Virginia and was successful in Ontario only when mature clumps of plants were moved into the garden. The species has been studied in the field at Sauble Beach on Lake Huron, Gray County, Ontario, and at Turkey Point on Lake Erie, Norfolk County, Ontario. Considerable variations are noticeable in the field, and at Turkey Point seeds were collected from both linear-leaved plants and plants with ovate to elliptical leaves. Fernald (1911) discussed the variations occurring in North America and described four varieties in addition to the type. The chief variations are leaf size and shape, shape and size of the stem, and degree of

pubescence. The gametic chromosome number was determined as $n=7$ (fig. 26).

L. ochroleucus Hook. $2n=14$.—White (1894) gives the distribution of this species as "New York and New Jersey, north and westward to Washington and British Columbia." Raup (1936), discussing the flora of the Athabaska-Great Slave Lake region of northern Alberta, says *L. ochroleucus* Hook. is "abundant in the prairies, clearings, and dry open woods northward at least to the Wood Buffalo Park and noted in Fl. Bor. Am. as extending to Great Bear Lake. Scarcely known east of the Paleozoic boundary."

This species has also proved difficult to establish in experimental plots. It has been found and studied in the field on the Six Nations Indian Reservation, near Hagersville, Ontario, and in Norfolk County, several miles back from Lake Erie. In the first location the plants were growing on a rather dry heavy clay bank about 15 or 20 yards above a stream. In the second location the plants were found in dry sandy open woodland. The plants found at each station were very similar. The upper epidermis is remarkable in the genus for the almost complete absence of stomata. The cells are somewhat variable in shape, some of them being as broad as long. The walls have fairly deep undulations. The somatic chromosome number of the material from Hagersville was 14. This species has not previously been assigned to a section of the genus, but the material studied clearly indicates that it should be included in the *Orobastrum* section.

L. venosus Muhl. $n=14$, $2n=28$ (fig. 9, 10, 30, 31, 50).—Butters and St. John (1917) pointed out that this species consists of at least three varieties which are fairly well delineated geographically and morphologically. The type variety is nearly glabrous, has ovate-lanceolate stipules, elliptic or ovate-elliptic leaflets and is found entirely in the Appalachian region. Variety *meridionalis* Butt. and St. John is lightly pubescent, has linear-lanceolate stipules, ovate leaflets, and occurs in the southern Appalachian region and in Texas and Louisiana. Variety *intonsus* Butt. and St. John is pubescent throughout, has linear-lanceolate stipules, elliptic leaflets, and is distributed from the Appalachian region through the Great Lakes district to the Dakotas and Alberta. The culture 55.1, which came from Edmonton, Alberta, and was studied cytologically, was markedly pubescent and should be considered var. *intonsus*. The other culture, 55.6, which has been studied cytologically, came from the Massanutten mountains of Virginia and has characteristics of both the type variety and the variety *meridionalis*. This culture was studied in the field as well as in experimental plots. It occurs in rocky woodland and in a few isolated spots, but is not at all common in the region in which it was found.

The epidermal cells of the two cultures studied are similar, except that those of 55.6 have fairly deep undulations in the walls, whereas those of 55.1 (fig. 55) have almost no undulations. In general, the cells

occupy a fairly large area, being broad, although they are not nearly so long as the epidermal cells of some species.

Material of both 55.1 and 55.6 was examined cytologically and the gametic chromosome number determined as 14. Only small amounts of this tetraploid have been available thus far, so it has not been possible to study the meiosis in as much detail as is desirable. Examination of smears has consistently shown irregularity of the I and II metaphases and resulting abnormal tetrads and pollen. In some cells 14 bivalents were found (fig. 31), while in others 12 bivalents and 4 univalents were observed (fig. 30). Fourteen chromosomes were counted in II M of 55.1. Tetrads with 2, 4, 5, and 6 cells were found in 55.1. Examination of 1806 pollen grains showed 19.42 per cent of badly formed, probably non-viable, pollen. The plants which have been studied have set little or no good seed thus far. Observations in the field indicated that little good seed was set there. The somatic chromosomes are difficult to count accurately, since they are large and the cells are crowded. However, counts were made on both 55.1 (fig. 50) and 55.6, and the number $2n=28$ was found. This is a most interesting species, since it is the only one out of 42 species of *Lathyrus* thus far studied by the writer and others which normally has a chromosome number other than $n=7$. *L. venosus* Muhl. has not previously been assigned a place in the arrangement of the genus. Its morphology indicates that it should be placed in the *Orobastrum* section.

L. setifolius L. $n=7$.—This small annual species occurs through southern Europe from Portugal and Spain to Asia Minor and Crimea. One culture was studied and carefully identified. The gametic chromosome number was determined as $n=7$ (fig. 27).

L. sphaericus Retz. $n=7$.—This is also an annual species and is found in the Madeira Islands and the Mediterranean region of southern Europe and northern Africa east to the Caucasus. Three cultures were grown and the gametic chromosome number determined as $n=7$ in a culture from Coimbra (fig. 29). This culture was identified as *L. sphaericus* L. var. *stenophyllus* Boiss., following Ascherson and Graebner (1906-1910).

L. inconspicuus L. $2n=14$.—This species is a slender annual which is distributed throughout the Mediterranean region. One culture was grown and the somatic chromosome number determined as $2n=14$ (fig. 45).

L. nigribalvis A. Burkart $n=7$, $2n=14$.—This is an annual or biennial species occurring in northern, subtropical Argentina. The culture studied was grown from seeds from the type culture of this species. The style resembles those of the *Chymenum* section somewhat, but the leaf characters clearly indicate that this species should be included in the *Orobastrum* section. Gametic and somatic chromosome numbers were determined ($n=7$, fig. 28, $2n=14$).

Section *Orobis* (Tourn.) Taubert

The species of this section are characterized by an erect rather than a climbing habit and by the leaves being bristle tipped rather than tendrilled.

L. niger (L.) Bernh. $n=7$, $2n=14$ (fig. 8, 12, 32, 56).—This is a perennial species which is found in Algeria and in Europe east to central and southern Russia and the Caucasus. Three cultures were grown and the chromosome numbers determined as $n=7$ (fig. 32) in two of them and $2n=14$ in the other.

L. vernus Bernh. $2n=14$.—This is also a perennial species which is distributed through Europe to western Siberia, southern Russia, and the Caucasus. One culture was grown, and the somatic chromosome complement was found to consist of 1 pair of long submedian chromosomes with a secondary constriction in the longer arm, 1 pair of long submedian chromosomes with an arm ratio of 3:8, 2 pairs of long submedian chromosomes with an arm ratio of 1:4, 1 pair of medium median chromosomes, and 2 pairs of short median chromosomes.

Section ?

The following species have never been assigned to sections of the genus and until further studies are made must be merely listed here. The chromosome counts on South American species were made from root tips grown from seeds of plants collected and identified by Dr. Arturo Burkart.

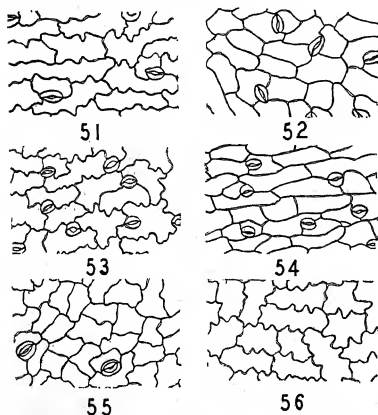


Fig. 51-56. Epidermal cell patterns of *Lathyrus*; camera lucida drawings of the upper leaf epidermis, magnification $\times 120$. Section *Cicerula* Moench.—Fig. 51. *L. sativus* L. Section *Eulathyrus* Ser.—Fig. 52. *L. latifolius* L. Section *Nissolia* Tourn.—Fig. 53. *L. Nissolia* L. Section *Clymenum* DC.—Fig. 54. *L. Clymenum* L. Section *Orobastrium* Gren. and Godr.—Fig. 55. *L. venosus* Muhl. Section *Orobis* (Tourn.) Taub.—Fig. 56. *L. niger* (L.) Bernh.

L. crassipes Gillies $2n=14$ (fig. 49).—This is an annual species occurring in Brazil, Paraguay, Uruguay, Argentina, Chile, and Peru. The material was collected by Burkart at Santa Fe, Argentina.

L. dumetorum Philippi $2n=14$.—This perennial species occurs in central Chile and west central Argentina. Seeds were collected at Neuquén, Argentina, on Lake Nahuel Huapi.

L. macropus Gillies $2n=14$.—This is also a perennial species found in northwestern Argentina and in Chile. The material studied came from Tucumán, Argentina. According to Burkart (1935) this is the Argentine species which shows the most affinity to the species of western North America.

L. paranensis A. Burkart $2n=14$.—This is an annual species occurring on the shores of the La Plata river, Argentina. Chromosome counts of $2n=14$ were made on two cultures, both of which came from the Delta del Paraná, Argentina.

L. Parodii A. Burkart $2n=14$.—This perennial species also is found on the delta of the La Plata river in Argentina. The material studied came from Delta del Paraná, province of Buenos Aires, Argentina.

L. quadrimarginatus Bory and Chaub. $2n=14$.—This is a small annual species occurring in Greece. One culture from Coimbra was grown and identified and the chromosome number determined as $2n=14$.

L. sessilifolius Hook. and Arn. $2n=14$.—This species is a perennial which is found in Chile, the Andes of Argentina, and through central Argentina to the province of Buenos Aires and Uruguay. Two collections were studied cytologically, both from Neuquén, Argentina, on Lake Nahuel Huapi.

DISCUSSION.—Since of 42 species studied, 41 have $n=7$, chromosome numbers in *Lathyrus* are of little assistance in devising a natural arrangement of the species. The general similarity in size of the chromosomes and of the position of their spindle-fiber constrictions also precludes to a considerable extent the use of chromosome morphology in the evaluation of species relationships. The section *Clymenum* DC. is, however, sharply characterized cytologically by the presence of two small pairs of chromosomes in all three species of the section (fig. 24, 25, 45, 46). The epidermal cell-pattern of the leaf of the various species show more uniformity within the limits of the sections as established by Ascherson and Graebner (1906-1910) than intersectionally. Space has not permitted the presentation of drawings of the calyx and pistil of each of the species studied nor of the detailed measurements of the various organs of the living plants. The calyces and pistils of the various species showed little individual variation, small specific differences, and considerable uniformity within the limits of Ascherson and Graebner's sections. The section *Clymenum* DC. is again sharply delimited by its cylindrical calyces with truncate bases and its spatulate styles. Chromosomally and morphologically this section constitutes a well defined group of closely related species.

Each of the species studied critically morphologically came within the limits of one of the sections of Ascherson and Graebner, indicating that these sections are valid for at least a part of the American species as well as the species of Europe and Asia. The analysis of geographic distribution has shown the primary center of development of the genus to be in the Mediterranean region with outlying secondary centers in western North America and temperate South America. All the Old World species studied are diploids while the one tetraploid found occurs in North America toward the periphery of the generic range. This is in accord with the distribution of polyploidy in other genera of Old World origin. In *Crepis* the Old World species are predominantly diploid and the New World species polyploid (Babcock and Cameron, 1934). Similarly, Sax (1936) found most of the Old World species of *Spiraea* to be diploids and the American species to be tetraploids.

SUMMARY

Few or no successful interspecific crosses have been made in *Lathyrus*. In the present investigation 458 attempts at interspecific crosses yielded seed in only

4 instances, one lot of which failed to germinate, and the other three of which produced plants entirely maternal in appearance, indicating that contamination had occurred.

Chromosome number determinations on the thirty-five species indicate that all are diploid ($n = 7$, $2n = 14$) with the exception of *L. venosus* Muhl. which is a tetraploid ($n = 14$, $2n = 28$). Comparative morphology of the species studied, morphology of the pistils and calyces, and epidermal cell patterns support Ascherson and Graebner's arrangement of the species of the genus. Lack of positive evidence from interspecific hybridization and the great uniformity of chromosome number make the determination of the evolutionary history of the genus difficult. *L. ochroleucus* Hook., *L. venosus* Muhl., and *L. nigri-valvis* A. Burkatt should be assigned to the *Orobatrachum* section and *L. numidicus* Batt. to the *Cicerula* section. The section *Clymenum* DC. is sharply defined cytologically as well as morphologically.

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TRANSFORMATION OF SUGARS IN SUGAR BEET AND CORN LEAVES AND INVERTASE ACTIVITY¹

O. A. Leonard

THIS STUDY was undertaken to determine the possible transformations which may occur when glucose, fructose, and sucrose are supplied separately to sugar beet and corn leaves. An understanding of the transformations of the sugars is necessary before data on photosynthesis, translocation, respiration, growth, storage, and other physiological processes in which sugars are involved can be evaluated.

Virtanen and Nordlund (1934) state that while it is known that fructose is converted into glucose in the mammalian body, evidence that the same change occurs in the plant world is lacking. They found that wheat and red clover leaves placed in either glucose or fructose solutions formed an abundance of sucrose. They concluded that there was some conversion of fructose into glucose and vice versa. Nurmin (1935) found fructose and glucose were readily interconverted and that this interconversion was accompanied by a synthesis of sucrose. The blades of red clover (*Trifolium pratense*) and the horse bean (*Vicia faba*) were found to be free or almost free of invertase and yet sucrose was formed from either glucose or fructose. Evidently the sucrose in the blades was not formed by invertase but, as Nurmin suggested, by a sucrose-synthesizing system. The synthesis of sucrose from glucose and fructose requires energy which is probably obtained through respiration.

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The author wishes to express his thanks to Dr. W. E. Loomis of Iowa State College for helpful suggestions during this investigation.

Hartt (1937) reported the results of experiments with detached sugar cane leaves. She found that either glucose or fructose were transformed into sucrose when supplied artificially to sugar cane leaves. She observed that the synthesis was most complete when low concentrations of glucose and fructose were supplied. In a recent paper (Leonard, 1936) the author noted that the relative proportions of sucrose and the reducing sugars were associated with the water content of the tissues.

MATERIALS AND METHODS.—Recently matured leaves of corn and sugar beets were removed from the plants, and the bases of these were placed in 3 and 6 per cent sugar solutions. Check samples were taken at once. The blades of the leaves were left exposed to the air so that transpiration might draw the solutions up into the xylem of the leaves. In some experiments the blades were submerged in sugar solutions. Samples of leaves were rinsed with distilled water and then dropped into 450 cc. of boiling 95 per cent alcohol and allowed to simmer for 20 minutes. The sugars were extracted and analyzed following the methods listed in Loomis and Shull (1937). The Munsen-Walker-Bertrand method was used for determining reducing sugars and sucrose, and Jackson and Mathews' modification of Nyn's method for fructose. Sucrose was inverted by invertase. All of the dextrin analyzed was soluble in 10 per cent alcohol. The green weight of the sugar midribs and blades was recalculated from the extracted dry weight. The corn data are placed on the extracted dry weight basis.

Other feeding experiments were attempted. The blades of entire sugar beet leaves were floated on sugar solutions, the petioles being placed in distilled water. Under these conditions there was no absorption of

TABLE 1. *Changes in the composition of blades of sugar beets floated on sugar solutions from 5 a. m. to 5 p. m. September 9, 1936.*

	Glucose		Fructose		Sucrose		Dextrin	
	Per cent	Increase	Per cent	Increase	Per cent	Increase	Per cent	Increase
Initial composition ...	0.18		0.18		0.12		0.03	
2 per cent fructose	0.29	0.11	0.15	-0.03	0.17	0.05	0.42	0.39
2 per cent glucose	0.41	0.23	0.12	-0.06	0.17	0.05	0.43	0.40

sugar. Either the external leaf membranes were non-permeable to the sugars or the sugars were prevented from entering by the cuticle. If the external membranes were impermeable to the sugars, there is a difference in permeability between these membranes and the membranes of the cells which surround the xylem. The latter cells were permeable to all the sugars tested.

Slices of beet roots, also, were placed in fructose and glucose solutions of various strengths, with and without forced aeration. The analyses of the treated material did not differ significantly from the original composition of the roots. We conclude that the cells of the root are not readily permeable to sugars.

is usually transformed more readily into glucose than is glucose into fructose. In other feeding experiments the same transformations were observed.

Sucrose was hydrolyzed approximately as rapidly as it was absorbed, and the sucrose concentration was about the same whether the leaves were fed glucose, fructose, or sucrose. Invertase, as will be shown later, was very active in sugar beet blades and hydrolyzed most of the sucrose which was absorbed. There was a sucrose-synthesizing system. Nurmia (1935) showed that the leaves free or almost free of invertase were able to synthesize sucrose readily from either glucose or fructose. In the sugar beet root invertase is absent, and it is probably here that the

TABLE 2. *Composition of sugar beet blades and midribs before and after being fed sugar. May 3-5, 1937.*

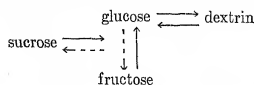
	Glucose		Fructose		Sucrose		Dextrin	
	Per cent	Increase	Per cent	Increase	Per cent	Increase	Per cent	Increase
Initial composition								
Blades	0.17		0.08		0.20		0.18	
Midribs	1.03		0.00		0.30		0.26	
3 per cent fructose								
Blades	0.23	0.06	0.25	0.17	0.29	0.09	0.32	0.14
Midribs	1.63	0.60	0.39	0.39	0.69	0.39	0.38	0.12
3 per cent glucose								
Blades	0.30	0.13	0.22	0.14	0.35	0.15	0.34	0.16
Midribs	1.14	0.11	0.10	0.10	0.46	0.16	0.34	0.08
3 per cent sucrose								
Blades	0.55	0.38	0.40	0.32	0.31	0.11	0.49	0.31
Midribs	1.40	0.37	0.35	0.35	0.56	0.26	0.39	0.13

PRESENTATION OF DATA.—*Sugar beet leaves.*—It can be seen in table 1 that fructose was readily converted into glucose. In fact, in this experiment there was actually a decrease in the percentage of fructose, even though fructose was being absorbed abundantly. Apparently the dextrin-synthesizing system was active, since considerable quantities of dextrin were formed. The sucrose response was not great, but here, too, there was synthesis.

In another experiment, as shown in table 2, the midribs also were analyzed. Whereas in the former experiment there was no evidence of a glucose to fructose transformation, this evidence was obtained in the latter experiment. In sugar beet leaves fructose

final traces of fructose combine with glucose to form sucrose. The author has never observed fructose to be present in the root of the sugar beet (unpublished research). Evidently, contrary to the general supposition, sucrose is not synthesized by invertase, but by some other system.

A diagram illustrating the observed sugar transformations in the sugar beet leaves is shown below.



Corn leaves.—Four feeding experiments were conducted on corn leaves. The results of these experiments are shown here. The leaves were left in the sugar solutions for 24 hours. In table 3, the leaves were fed 3 per cent sugar solutions and in table 4, 6 per cent solutions. As with sugar beets, fructose and glucose were interconverted, and again fructose was transformed into glucose more readily than was glucose into fructose. Glucose was transformed into dextrin. Dextrin which was observed to accumulate

In experiments not reported here corn leaves were placed in tap water for 24 hours after they had been fed sugar solutions for the same period of time. The sucrose/reducing sugar ratio was approximately the same as in the data reported here. This gives added weight to the view that equilibrium was very quickly established.

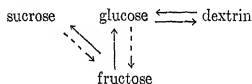
The observed reactions which occurred in the corn leaves are shown below. The relative activities of the dextrin-synthesizing and hydrolyzing systems are not

TABLE 3. *Composition of corn leaves before and after being fed 3 per cent sugar solutions. Calculated as percentage of the extracted dry weight.*

	Glucose	Fructose	Sucrose	Dextrin	Sucrose/Reducing sugar
Initial composition ..	1.02	0.11	2.03	0.90	1.79
3 per cent fructose ...	1.77	0.70	6.63	1.70	2.68
3 per cent glucose ...	2.26	0.36	7.09	2.40	2.70
3 per cent sucrose ...	1.82	0.39	6.52	2.65	2.95

was soluble in 10 per cent alcohol. In the feeding experiments there was no significant increase in the acid-hydrolyzable material. The synthesis of sucrose was considerably closer to being complete in the corn leaves than it was in the sugar beet leaves. The greater synthesis of sucrose than was observed in the leaves of the sugar beet is correlated with the weaker invertase activity in the corn leaves. The sucrose/reducing sugar ratio was about the same, whether the leaves were fed glucose, fructose, or sucrose. The initial ratio is the lowest, probably because the distribution of the sugars within the cells was different than at the higher sugar concentrations. It may be observed that sucrose increased approximately 6 times its original concentration (table 4). The reducing

known, since no dextrin feeding experiments were successful. The dextrin solutions clogged the xylem and resulted in desiccation of the leaves by transpiration.



Dandelion leaves.—Feeding experiments likewise were carried out using dandelion leaves. As will be shown later, dandelion leaves are very low in invertase, being in this respect lower than corn. Again fructose and glucose were interconverted and again

TABLE 4. *Composition of corn leaves before and after being fed 6 per cent sugar solutions. Calculated as percentage of the extracted dry weight.*

	Glucose	Fructose	Sucrose	Dextrin	Sucrose/Reducing sugar
Initial composition ..	1.02	0.11	2.03	0.90	1.79
6 per cent fructose ...	3.34	1.00	11.43	1.97	2.66
6 per cent glucose ...	3.49	1.34	12.00	4.10	2.48
6 per cent sucrose ...	3.28	1.00	12.31	2.00	2.83

sugar response was slight in comparison to that of sucrose. Barr (1933) found that when photosynthesis was active, sucrose increased markedly, while the reducing sugar response was small in comparison. Evidently, we may observe the same response whether the sugars are formed in photosynthesis or introduced artificially. Sugar transformations within the tissue prevent the use of diurnal accumulations as evidence that the particular forms are produced in photosynthesis or are moved in a given transport system. When, as our data indicate, even fructose and glucose are readily interconverted and vary with the tissue and its condition, we can hardly expect to determine the sugar formed, moved, or used in a particular case by simple chemical analyses.

the apparent equilibrium point was closer to glucose than it was to fructose, the same as it was in corn and sugar beet leaves. A large portion of the glucose and fructose absorbed by the leaves was synthesized into sucrose. Leaves of dandelion plants were placed in diffuse sunlight in order to observe their response to photosynthesis (table 5). The sucrose response in the blades was marked, the same as was observed in feeding experiments, while the reducing sugar response was slight. The sugar transformations which occurred in the blades were about the same as occurred in the corn leaves.

It is of interest to note that the sugars increased very markedly in the petioles. Glucose, for instance, increased about 8 times its original concentration.

The sugars moved from the blade mesophyll into the petiole in a polar direction. This movement was first shown by the author (Leonard, 1937) to occur on detached sugar beet leaves.

The invertase activity in the dandelion petioles was greater than it was in the blades. The greater invertase activity was associated with a greater proportion of sugars being in the inverted form. Factors other than invertase activity alone, however, are operative in determining the sucrose/reducing sugar ratio. In another paper (Leonard, 1936) the author has shown that the content of water in a tissue affects this ratio. The more hydrated tissues have a greater proportion of their volume occupied by vacuoles and relatively less by protoplasm. If invertase is localized mainly in the vacuoles and the sucrose-synthesizing system

in interpreting sucrose and reducing sugar levels in plants. It seems apparent that sucrose is not synthesized by invertase, except for the slight amounts of sucrose which are formed when invert sugars and sucrose are in equilibrium. Nurmia (1935) demonstrated that sucrose accumulated in the blades of red clover and horse beans in the absence of invertase when the blades were fed solutions of either glucose or fructose.

In table 6 may be seen the invertase activity in different parts of the sugar beet plant. The invertase activity is greatest in the blades and decreases from the upper part of the petiole towards the base. The usual sucrose gradient in the petioles is opposite to the gradient of invertase activity. The activity was not markedly different in blades of different ages, but

TABLE 5. Response of detached dandelion leaves to photosynthesis. Leaves placed in water and moved to diffuse sunlight for 12 hours.

	Glucose		Fructose		Sucrose	
	Check	12 hrs.	Check	12 hrs.	Check	12 hrs.
Blade	0.07	0.14	0.06	0.08	0.24	0.66
Petiole	0.06	0.50	0.06	0.10	0.12	0.32

in the protoplasm, then to increase the volume of the vacuoles, the volume of the protoplasm remaining constant, should affect the sucrose/reducing sugar ratio. Dandelion blades are more hydrated than corn blades, and this hydration probably is involved in keeping fair amounts of reducing sugars in the blades.

Araucaria excelsa needles were found to contain no active invertase. Invert sugars in the needles were confined to fructose. Evidently, the glucose-fructose reaction proceeded towards the right but not to the left. Fructose was not used in the formation of sucrose because there was no glucose to combine with it.

Invertase activity.—Invertase activity was studied in the hope that the information obtained might aid

in the petioles it decreased markedly with age.

The distribution of invertase activity in dandelion blades and petioles is different than it is in the sugar beet. The sugar beet blades have a greater invertase activity than the petioles, while the reverse is true for dandelions. The activity in the blades of the dandelion is very low in comparison to that in the sugar beet. There is less difference in the activity of the invertase in the petioles. In red clover, Nurmia (1935) noted that invertase activity was greater in the petioles than in the blades, as the author observed in the dandelion.

Corn (Barr, 1933) and oats (Nurmia, 1935) accumulate mainly sucrose within their blades, while sugar beets accumulate mainly the reducing sugars. The

TABLE 6. Invertase studies. Six-tenths grams tissue ground in a mortar with sand and made up to 250 cc. with a 2% sucrose solution. Results expressed in mg. invert sugar per 100 cc. solution. Toluol was added to the solution.

	0 hrs.	12 hrs.	36 hrs.	60 hrs.
Sugar beet				
Mature leaf				
Blade	0.0	111	448	712
Upper-petiole	0.8	43	85	100
Middle-petiole	0.8	39	70	91
Lower-petiole	0.8	20	37	49
Crown	0.0	14	18	32
Root	0.0	0.0	0.0	0.0
Corn leaves	0.0	36	80	115
Oat leaves	0.0	44	73	97
Dandelion leaves				
Blade	0.0	—	—	35
Petiole	0.0	—	—	70

invertase activity of the former is lower than for the sugar beets and may be responsible for the differences noted between these plants. However, more than one system conditions the reducing sugar and sucrose levels and may affect the results, regardless of the invertase activity.

DISCUSSION.—The difference in permeability of the external membranes and those surrounding the xylem cells enables sugars to be absorbed when the cut petioles are placed in sugar solutions but to be excluded when the uncut blades are placed in the same solutions. The significance of this difference in permeability is conjectural. In the springtime the sugar maple loses sugar to the xylem elements, but this sugar is quickly reabsorbed as soon as growth commences. This again illustrates the permeability to sugar of the membranes surrounding the xylem.

Membranes other than the peripheral membranes likewise are impermeable to sugars. Slices of roots exposed to fructose and glucose solutions of different concentrations showed no sugar absorption. Apparently the permeability of the membranes which surround the xylem represents an anomalous condition.

Known sugars introduced into plant tissues undergo marked transformations. Fructose and glucose are readily interconverted. Glucose may be condensed to form dextrin. Glucose and fructose combine to form sucrose. Sucrose added to such tissues undergoes partial hydrolysis to form fructose and glucose, and these sugars again undergo transformations. In the study of photosynthesis, one cannot know the first sugar of photosynthesis on the basis of sugar fluctuations within the blades. Hartt (1937) fed sugar cane leaves glucose and fructose sugar solutions and analyzed these leaves after intervals of time. She noted that the sucrose content rose markedly, while the reducing sugar response was slight. Evidently, in studies on translocation where more than the phloem is analyzed one cannot be certain whether the fluctuations in the sugars represent the sugars being moved through the plant, or whether they merely represent accumulations of non-motile sugars, which were manufactured in place from motile forms.

In the sugar beet the hydrolysis of sucrose is associated with the very active invertase present in the leaves. Corn and dandelion leaves, having a lower invertase activity than sugar beet leaves, hydrolyzed only a small portion of the sucrose absorbed by them. Sugar beet leaves synthesized only small quantities of sucrose, while corn and dandelion blades synthesized it abundantly from either glucose or fructose. Broadly speaking, the synthesis of sucrose is negatively correlated with invertase activity and indicates that

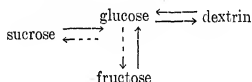
sucrose is synthesized by some other system than invertase.

There are many factors which should be noted when considering the relative amounts of the various sugars in tissues. Some of these factors are as follows: (1) the activity of the various carbohydrate transforming systems, (2) distribution of these systems within the cells, (3) distribution of the carbohydrates within the cells, (4) time available for the reactions, (5) temperature, (6) growth and respiration, and (7) translocation.

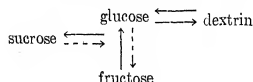
SUMMARY

Sugar was not absorbed through external leaf membranes, nor through the internal membranes of root cells. Sugars were absorbed through the membranes surrounding the xylem cells. Evidently the cell membranes surrounding the xylem are permeable to sugars and the other plant membranes are not.

In feeding experiments, the following diagram represents the sugar transformations which were observed to occur in sugar beet blades (with the exception of dextrin hydrolysis).



The diagram shown below represents the sugar transformations which were observed to occur in corn leaves (with the exception of dextrin hydrolysis).



The major portion of the sucrose absorbed by the sugar beet blades was hydrolyzed, while in corn leaves only a small portion of it was hydrolyzed. The hydrolysis of sucrose was associated with relatively great invertase activity. The slight hydrolysis of sucrose in corn and dandelion blades was associated with relatively weak invertase activity. This correlation is taken as evidence that sucrose is hydrolyzed, but not synthesized by invertase. It is suggested that sucrose synthesis is brought about by a sucrose-synthesizing system existing, perhaps, on protoplasmic surfaces.

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LEAF TEMPERATURES¹

Raymond H. Wallace and Harold H. Clum

THERE HAS long been a moot question in physiology as to the exact function of transpiration in the economy of the plant. Some have maintained that it is merely a necessary evil, while others have attributed to it many beneficial effects, including the cooling of leaves. Thus, in recent years, Miller and Saunders (1923), Eaton and Belden (1929), and Copeland (1932) have found an apparent inverse ratio between leaf temperatures and transpiration rates, to which they have attached more or less importance. On the other hand, Clum (1926) reduced transpiration and observed only small increases in temperature as compared to temperature changes produced by other factors; and, finding no constant relation between such temperature differences and the transpiration rates as measured, concluded that the cooling effect of transpiration must be insignificant. Curtis, in a number of papers (1926, 1936a, 1936b) has argued that transpiration as a cooling agent is not as important as often claimed; and Watson (1934) calculated, from the rate of cooling of leaves in still air, from transpiration rates, and from other factors, that re-radiation often accounts for more than 50 per cent of the heat loss from leaves. The present study is a detailed re-examination of the problem of leaf temperatures with more adequate measuring devices and new technique.

All plants used in this work were growing in the garden or about the lawn around the laboratory and should represent very well, therefore, what is taking place in a normal environment. Only the supplementary tests on leaf transmissions and reflections were run on detached leaves. The experimental period was from June 15 to Sept. 1, the period when plants are most active photosynthetically. The experiments were supervised almost twenty-four hours a day, the laboratory itself being an apartment over a triple garage at the home of the one author at Storrs, in which the other author lived while the experiments were in progress.

The duplex recording potentiometer (Wallace, 1937a) used in studies on methods of light sampling is the fundamental basis of this study. It was obvious from the first few records in the present study that hand readings are unreliable and subject to serious errors

of interpretation because of the very great and rapid temperature changes which are normal to leaves. The use of the duplex recorder eliminates this, however, since one can get two continuous records, from one leaf or from two leaves or any other combination desired. And, as was the case on light sampling, when one measures the same leaf at the same time with two bridge circuits which are identical but separate and obtains two records that are alike, one knows that conditions are under control. Full details of this instrument are given elsewhere, so that we will give here only those modifications necessary to adapt the instrument to thermocouple work. It will be observed below that in many cases three simultaneous records were made. The third record was from a single recorder of the same type, which was also available. The thermocouples were of copper and constantan (number 38 wire) made up according to Clum (1926a). It was found desirable to use more than a single couple, so thermopiles of five couples each were used in all tests. Surprisingly little difficulty was experienced in breakage of these couples. It seems unnecessary to use such small wires, however. The five warm junctions of each unit were mounted on a wire clip within a six mm. square frame. When this clip was opened and pushed across the leaf and allowed to close, the five junctions were pressed up against the lower surface of the leaf and held in place by a similar frame pressing down from the top. In many of the early experiments, the junctions were inserted into the leaf tissue itself, as Clum (1926a) did in his previous work. Later this was found to be unnecessary, and the more simple method was used. Unless otherwise stated, it is to be understood that all junctions were in contact with the lower side of the leaf.

It will be recalled in referring to the duplex recorder (Wallace, 1937a, page 490) that when switch *Q* is closed and resistance *R* turned to full resistance, we have the minimum millivoltage on the bridge and that this is about 30 mv. The five junction thermopiles, on the other hand, will give about 5 mv. for 40°C. difference in temperature between the junctions. For this reason, an additional 75,000-ohm resistance was added in series with *R*, and the new range of the bridge became about 7 mv., which was very good for this work.

¹ Received for publication October 9, 1937.

The thermopiles and the recorder were calibrated as a unit. The cold junctions were placed in an ice-water bath, whereas the warm junctions were placed in a thermos bottle of water at 20° or 25°C. The recorder was then allowed to run until it came to balance. The distance in mm. that the traveling arm moved divided by the temperature difference between the two sets of junctions gives the number of millimeters per degree. The actual values of the three bridges (the one of the single and the two of the double recorder) were 4.30, 3.95, and 3.95, respectively. The value, 4 millimeters equal 1 degree Centigrade, is the calibration that was then used for all.

EXPERIMENTS AND RESULTS.—General relation air-leaf temperature.—At the beginning of these studies a continuous record of leaf and air temperatures from July 2 to July 9, inclusive, was run on *Heliospis*

evening approaches, until at 8 p.m. the leaf is just 2°C. below air.

This curve shows the greatest cooling of the leaf for the period of test, but not the greatest heating. On the preceding day, July 3, the leaf at one time was 4°C. above the air. Very frequently from midnight to 8 a.m., as shown in this curve, the leaf is approximately at air temperature, but this is by no means always the case, since other curves have shown the leaf to be two or three degrees below air all night. Perhaps the most interesting point about *Heliospis* is that it normally wilts every day and that the greatest cooling of 7°C. below air coincides with the period of most severe wilting. The wilting on this particular day became noticeable at 11:30 a.m. and grew progressively more severe until it reached its maximum at 2 p.m. When the wilting is most severe, the leaves hang limply in a vertical position, and the absorp-

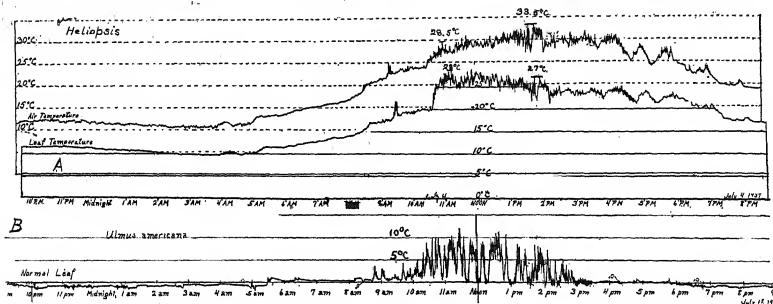


Fig. 1.—A. Air and leaf temperature curves for *Heliospis scabra* in terms of actual temperature. Cold junctions in ice water, warm junctions in air back of leaf for air temperature, and in contact with back of leaf for leaf temperature.—B. Air-leaf temperature of *Ulmus americana* obtained by suspending the cold junctions in the shade back of the leaf and attaching warm junctions to the lower surface of the leaf. See text for full details.

scabra, to obtain a general survey of the temperature relation. These curves were obtained for the leaf by attaching the warm junctions to the lower side of the leaf, as mentioned above, while the cold junctions were in an ice-water bath. The air temperatures were determined in the same manner, but with the warm junctions suspended in the air directly behind and in the shade of the leaf.

In figure 1A is given the record for July 4, a typical day for this period. It will be noted that the air and leaf temperatures run close together during the night, but that when morning comes (8 to 10:30 a.m.) the leaf drops four degrees below the air. There is then a sudden jump of the leaf temperature up to that of air at 11 a.m., when the direct rays of the sun hit the leaf. This is then followed by a period from 11 a.m. to 2 p.m., when the air temperature climbs higher and higher to reach a maximum of 33.5°C., while the leaf temperature drops lower and lower, reaching a minimum of 7°C. below air at 2 p.m. The two curves then draw closer together as

tion of energy from the sun is greatly reduced. This drooping may be of value in preventing excessive heating of leaves.

Examination of curve 1A will show that there may be, under some conditions, a very high degree of correlation between air temperature and leaf temperature. Note how each little detail or variation in temperature is present in both curves, although their amplitudes may be different. This point is very important because the leaf is reacting not in accordance with crudely integrated temperatures, as measured by thermometers, but is instead reacting to every rapid fluctuation, some of which doubtless escape even the recorder used here. When wind is blowing through the leaves, this correlation between leaf and air temperature all but completely disappears.

In view of the fact that determinations of leaf and air temperatures, as shown in figure 1A, used both sides of the duplex recorder, it was thought that perhaps one could show the same relation by merely attaching one set of junctions to the lower side of the

leaf and suspending the other set in the air directly behind the leaf. In this manner two simultaneous readings of leaf temperatures could be made. In figure 1B is shown such a curve for *Ulmus americana*. In this type of illustration one must remember that the horizontal line along the base of the curve represents 0°C. difference between leaf and air temperature. If the leaf is below air temperature, its curve passes below this line, while if it is above air the curve rises above this line. All subsequent curves in this paper are of this type.

One practical problem had to be overcome, however, in making these curves. It will be noted that whereas in the type 1A curve the sign of the potential never changes, since the leaf temperature is being measured against 0°C., and the leaf never gets that low, in the type 1B curve there are many and rapid changes in sign, as the temperatures of the leaf and air vary. This entails the use of the double bridge, or, in other words, the opening of switch Q (Wallace, 1937, p. 490). Examination of the diagram discloses that opening the switch automatically throws in two 25,000-ohm resistances in parallel, which is equivalent to adding 12,500 ohms in series with the null galvanometer. Then in addition one would have to add another 75,000-ohm resistance to balance the one used to adapt the bridge for the type 1A curve. This would of course destroy the sensitivity of the instrument, and render it unsuited to the work. One can, however, leave the bridge exactly as it was used for the type 1A curves above and add a small supplementary voltage from an external source in such manner that it adds to the voltage coming over from the thermopiles. This is done in the following manner: a 100,000-ohm fixed resistance is connected in series with a 100-ohm variable resistance, and the two ends of this resistance combination attached to the two terminals of a flash-light dry cell. If one now taps off the two ends of the 100-ohm variable resistance, he has a source of external voltage of from 0 to 1.5 millivolts to connect in series with the thermopiles, to take care of change in sign when the temperature of the leaf drops below that of air. On the other hand, the maximum resistance added in series with the null galvanometer is 100 ohms, which has very little effect on sensitivity. It might be well to mention that in this study the recorder has functioned very well on .01 millivolt, or .01 microampere, which means that the instrument responded to somewhat less than 1°C. difference in temperature. Also the variable resistance enables one to control the zero setting of the bridge. One merely shorts the leads to the thermopile and then adjusts the variable resistance until the traveling arm occupies the position on the bridge that one desires, and that becomes the position of the horizontal base line of 0°C. difference between leaf and air temperature.

Transpiring and non-transpiring leaves.—After examining carefully the normal fluctuations between leaf and air, which were recorded between July 2 and 9 on *Heliopsis*, it seemed obvious that the few degrees

above or below air which occurred were of very doubtful significance in the ordinary economy of the plant. The fundamental question then arose, "Are we primarily interested in these minor fluctuations, or might we better determine the temperature to which the leaf as a dark body will rise when the leaf is exposed normal to the sun, and transpiration is prevented?"

To determine this maximum temperature, leaves of *Heliopsis* were vaselined. The temperature promptly rose 5°C. above that for the non-vaselined leaf. The same test repeated the following day gave a rise of 7°C. above air. About 6 p.m. the direct rays of the sun no longer struck the leaf, and its temperature quickly dropped to 5°C. below the air. The story then became clear; transpiration was continuing in spite of the vaseline. The following morning the leaf remained 2°C. cooler than air until the direct rays of the sun struck it, and then it rose above air temperature. This same leaf was clipped from the plant at 2 p.m. and supported on a piece of white paper so that the sun would continue to strike it at the same angle, and in the next fifteen minutes it rose to 11°C. above air. A similar test an hour later with a normal leaf gave similar results, except that it rose to only 7°C. above the air.

It was obvious from these results that something other than vaseline must be used to get the temperature of a leaf as a non-transpiring, dark body. Two schemes were tested for this purpose. The first was to put a piece of clear photographic film over the lower surface of the leaf; this was sealed along the edge of the leaf with melted wax made of equal parts of beeswax and rosin. The second consisted of painting the leaf surface with the wax. A summary of these two methods at the end of the day showed that the leaf with the first method had an average temperature of 14.8°C. above air, while the second method gave 13.2°C. above air. This seemed to indicate no appreciable difference in the temperatures given by the two methods, but since the cellulose film method might interfere with connection currents, the wax method was chosen.

One must be very careful in using wax, however, because the leaves are easily killed, but if the wax is cooled until solidifying on top (about 62°C.) and then painted on the leaf, no injury results. Some leaves (*Forsythia*) completely covered on top and bottom, have remained apparently normal after being coated for two months. Other leaves, however, became mottled after a few days.

Orientation of leaf.—As has already been mentioned, the orientation of the leaf with respect to the sun may markedly affect the temperature which it will assume. This is well shown in figure 2A. The control leaf in this figure was quite normal to the sun, but the waxed leaf was oriented almost edgewise to the sun until artificially supported normal to the sun shortly after noon. The mere reorientation of this leaf resulted in its rising 11°C. higher than it had been. Prior to this, the waxed leaf was actually cooler without transpiration than the control leaf

normal to the sun with transpiration. It is to be assumed that all leaves in subsequent curves were held normal to the sun at noontime to insure the maximum heating of the leaves. This artificial orientation would seem to be entirely legitimate, since on any particular plant some leaves or parts of leaves will be in this position, and we would like to know if non-transpiring leaves in that position may be adversely affected by direct solar radiation.

mission and reflection. The transmission percentage of all leaves was determined both for visible and for total radiation from both artificial and natural sources. An apparatus was constructed consisting of a light-tight box with a window at the top 2.5 by 5 cm. For the measurements of light transmission a photonic cell was placed 20 cm. below the window and connected to a suitable millivoltmeter. For total radiation a Shirley radiometer (1930) placed close to

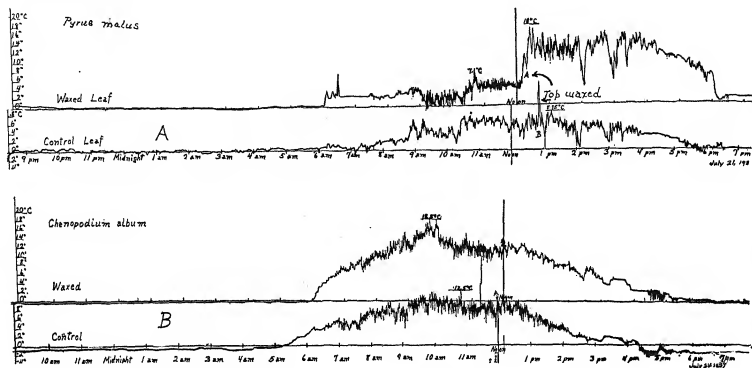


Fig. 2.—A. Waxed and control leaf-air temperature curves for *Pyrus Malus*. The waxed leaf was not normal to the sun until shortly after noon. A little later at "A" the upper surface of this leaf was waxed without affecting its temperature. The upper surface of the control leaf was waxed at "B," the hot wax causing a temporary peak in the curve.—B. Curves for waxed and control leaves of *Chenopodium album* showing the general temperature relation. The upper surfaces of both the waxed and the control leaves were painted black at "A," but this caused very little if any additional heating.

Arrangement of junctions.—It seemed possible that care should be exercised in the arrangement of the thermopile junctions about the leaf to insure adequate sampling of the temperatures. One method in use early in the study was to distribute the five junctions about the surface of the leaf. In figure 4A is shown a test of arrangement of junctions. It will be observed that the junctions in compact arrangement—that is, within the 6 mm. square frame already mentioned—showed a maximum temperature of 29°C., while those distributed about the leaf showed 27.75°C. In figure 4B the situation is just the reverse, in that the compact junctions show a maximum temperature of 21.8°C., while the scattered junctions show 23°C. There would seem to be no significant difference between the two methods, but because of ease of manipulation the compact arrangement was chosen.

Physical properties of leaves.—When wax was first used to prevent transpiration in order to get the heating of the leaf as a dark body, it seemed quite possible that corrections might have to be made to allow for the effect of the wax on the physical properties of the leaves. To determine this point, the twenty species of plants used were tested for trans-

mission and reflection. The transmission percentage of all leaves was determined both for visible and for total radiation from both artificial and natural sources. An apparatus was constructed consisting of a light-tight box with a window at the top 2.5 by 5 cm. For the measurements of light transmission a photonic cell was placed 20 cm. below the window and connected to a suitable millivoltmeter. For total radiation a Shirley radiometer (1930) placed close to the window in the box was substituted for the photocell. A variable resistance connected across the leads from the photocell or total radiation cell, in the manner described by Wallace (1937b), enabled one to control the deflection of the meter for ease of reading. For artificial radiation a 1000-watt bulb was suspended directly above the window of the box at a distance of 5 cm. The variable resistance was then adjusted to give a reading of 100 in the case of light, or of 30 spaces for total radiation. The leaf under test was then placed over the window and clamped in position. The drop in reading was noted and calculated as a percentage of the original reading to give relative transmission. The leaf was then immediately waxed, and a new reading made to obtain the effect of the wax on the transmissions. (See note on table 1.) The only modification necessary to adapt the instrument for use with natural radiation was to stand the box in the sun at high noon on a clear day and tilt it so as to bring the direct rays of the sun in the middle of the field where the photocell or total radiation cell was located.

In table 1 is given a compilation of these data for all species studied. In the first six columns the un-

TABLE 1. Physical characteristics of leaves.

Name of plant	Relative transmission percentages ^a										Relative reflection percentages ^a			
	Artificial radiation					Natural radiation					Artificial radiation			
	Visible		Total			Visible		Total			Visible		Visible	
	Normal	Waxed	Normal	Waxed	Vessel-ined	Normal	Waxed	Normal	Waxed	Vessel-ined	Normal	Waxed	Blackened	Vessel-ined
<i>Acer rubrum</i>	4.0*	2.0*	7.4*	21.0	21.0*	26.7*	1.7	1.1	18.4	13.8	11.6	16.6	13.0	13.0
<i>Acer saccharum</i>	2.8	2.3	10.0*	22.2	19.0	20.3*	1.0	7	13.9	7.0	10.0	20.6	17.0	17.0
<i>Athaea rosea</i>	2.6	1.7*	7.9*	21.1	20.3*	20.3*	1.1	7	14.6	11.4	14.1	16.2	17.5	17.5
<i>Asclepias syriaca</i>	3.6*	1.2*	5.0*	21.0*	20.0*	21.7*	1.1	9	13.9	14.1	13.3	24.1	1.5	10.5
<i>Chenopodium album</i>	4.9*	2.4*	7.9*	23.3*	22.7*	23.7*	1.7	1.3	16.9	16.4	17.9	17.2	7.0	18.5
<i>Cornus florida</i>	2.3	1.6	11.2*	20.3	19.0	26.3*	1.5	1.1	15.4	13.8	10.0	17.0	17.5	17.5
<i>Foreythia viridissima</i>	1.7	7	3.0*	18.3	15.0	21.0*	1.5	1.2	16.9	7.7	14.5	15.3	20.5	20.5
<i>Halimolobos scabra</i>	2.1	1.3	8.0*	21.5	18.3	22.7*	1.0	9	15.4	11.5	14.2	21.0	9.0	10.5
<i>Kalmia latifolia</i>	1.3	1.0*	2.8*	16.7	20.0*	21.7*	1.3	1.0	16.9	7.7	13.2	23.0	5.5	15.5
<i>Pyrus Malus</i>	2.5	1.5*	7.0*	21.0	20.0*	21.7*	1.0	1.1	15.4	13.8	11.0	17.0	10.5	10.5
<i>Phlox paniculata</i> var. <i>alba</i>	2.0	1.9*	6.5*	18.8	20.0*	22.7*	1.0	1.1	15.4	13.8	17.5	15.0	20.5	20.5
<i>Plantago major</i>	3.6	2.2*	9.0*	19.0	21.0*	23.3*	1.0	9	12.5	9.7	21.0	18.5	20.5	20.5
<i>Polygonum</i> sp.	7.0*	2.8*	9.5*	23.3*	20.7*	23.3*	1.5	1.0	13.8	16.6	13.0	18.0	12.5	12.5
<i>Pyrus communis</i>	7.0*	3.1*	6.3*	23.3*	21.7*	23.3*	1.5	1.1	15.4	13.8	13.7	16.5	27.5	27.5
<i>Rhus Toxicodendron</i>	7.6*	4.2*	12.0*	27.7*	23.3*	25.0*	1.5	1.3	16.9	13.8	13.0	18.0	24.5	24.5
<i>Smilax herbacea</i>	12.0*	5.5*	14.0*	28.3*	24.3*	27.3*	1.5	1.1	18.4	13.8	12.5	19.0	6.0	13.5
<i>Syringa vulgaris</i>	2.0	1.0	5.5*	19.4	17.7	22.3*	1.5	1.5	10.7	10.7	13.5	19.5	19.5	19.5
<i>Ulmus americana</i>	2.1	3.1*	6.8*	22.2	22.1*	22.7*	2.0	1.0	20.0	12.3	18.0	18.2	28.5	28.5
<i>Verbascum Thapsus</i>	11.5*	1.0*	11.7*	22.5*	14.3*	18.3*	2.0	1.0	20.0	12.3	18.0	16.0	22.5	22.5
<i>Vitis</i> sp.	4.0	3.2*	18.5*	21.0	21.2*	29.3*	1.4	1.0	15.9	12.2	14.2	18.5	5.6	17.9
Averages	2.4	1.4	8.5*	20.3	17.5	23.6*	1.4	1.0	15.9	12.2	14.2	18.5	5.6	17.9
	6.6*	2.6*		23.3*	20.7*									

^a The artificial radiation source was a 1000 watt Mazda bulb at a distance of 5 cm. above the leaf; the natural was the noonday sun. These data on relative transmission and reflection percentages are not intended to be quantitative studies on these physical properties. They are intended to show the general effect of wax on the leaves and should be treated as supplementary data.

* The values that are starred are determinations made August 23-26. Those that are unstarred were all made on July 15. The most important ones (for the visible and the total of sunlight) were made on July 15.

starred figures are readings made on July 15, while the starred ones were made on August 23. The averages given at the bottom of the table show clearly the relative effects of the various treatments on the physical properties of the leaves. Thus, beginning at the lefthand side of the table and reading across, we find the following averages: normal leaves showed a transmission in the visible of 2.4 per cent, while the same leaves waxed had a transmission of 1 per cent less. In the total radiation range the normal leaves transmitted 20.3 per cent, and after waxing the transmission dropped just 2.8 per cent. In like manner, the columns for the visible range with sunlight showed a drop from 1.4 per cent to 1.0 per cent on waxing, and the transmission of the total radiation from sunlight showed a drop from 15.9 for the normal to 12.2 for the waxed, or 3.7 per cent. It is obvious from these data that the maximum reduction in relative transmission for the leaves in this study when waxed is around 4.0 per cent. In other words, the waxed leaf should heat up not more than 4.0 per cent more than an unwaxed leaf, if transmission of total radiation were the sole factor involved. It is interesting to examine the various columns of both starred and unstarred figures and to see the marked increase in transmission which had already taken place by August 23. This change shows up about the same for both the visible and the total radiation range. Vaselineing the leaves causes an increase of about 2 per cent in transmission of the visible, but seems to have no effect on the transmission of total radiation. It is also evident that transmission for both the visible and the total radiation range is quite different for artificial and for natural radiation.

In the last four columns are given the values of relative light reflection from the leaves for various conditions. These readings were made by an instrument set up as follows. A 22-mm. projection lantern was so arranged as to focus a uniform field 1.5×2 cm. on one of the 2-inch faces of a cube of $MgCO_3$ eight inches from the lens of the lantern. This cube of $MgCO_3$ was mounted on a mechanical arrangement that enabled one to turn it on its vertical axis or swing it back and forth to bring the face of the cube into a perfect vertical plane. A high sensitivity photocell (Electrocell no. 782) was then mounted in a vertical plane about six inches in front of the face of the cube in such a manner as to make an angle of 15 degrees from the lantern lens to the cube face and back to the photocell. The photocell was connected to the meter and to the variable resistance as above for transmission. Readings were made as follows: First the block of $MgCO_3$ was set for vertical position and then the operator simultaneously watched the meter and turned the adjustment back and forth to swing the face of the cube on its vertical axis. The adjustment which gave the maximum reading on the meter was taken as maximum reflection of a surface. The variable resistance was then adjusted to make the meter read 100. A leaf

was then held against the surface of the cube and the reading made on the meter.

Referring again to table 1, it will be seen that the relative reflection from normal leaves ranged from a low of 10 per cent to a high of 24, with an average of 14.2. For the waxed leaf, on the other hand, the average reflection increased 4.3 per cent—that is, if reflection were the sole factor involved in the heating of a leaf, a waxed leaf should be 4.3 per cent cooler than a normal leaf. Vaselineing leaves increased reflection to about the same extent as waxing, but blacking the leaves with lampblack reduced the reflection 8.6 per cent.

It is probably not significant that on these twenty species the wax treatment of the leaves decreases relative transmission about the same percentage that it increases the reflection, thus theoretically leaving the energy relation unchanged. It does show, however, that in leaves that are waxed we do have these two physical properties working against each other, and that perhaps to some extent they do cancel. Leaves were painted black, which decreased their reflection 8.6 per cent, and still no marked effect on the height of the curves appeared. This is well shown in figure 2B for *Chenopodium album*. Both the waxed and control leaves were painted black at noontime at the points labeled "A." Neither curve shows an appreciable change from the treatment. Several additional tests on this point were made with like results. The lampblack was applied to the leaves both in water and in gasoline medium. When applied with water, no plant has ever shown injury from it, and many leaves are still entirely normal after having been blacked for two months. When applied with gasoline, on the other hand, only those leaves whose upper surfaces were entirely impermeable to the gasoline survived; the others died within a period of an hour or two. This seems to be a simple method of demonstrating the presence of stomata on the upper surfaces. Of the species studied, only maple, apple, pear, dogwood, and *Forsythia* survived the gasoline treatment.

Possible insulation effects from the wax treatment were also tested by means of mercury thermometers. In the first test two matched thermometers were placed horizontally below a 1000-watt Mazda bulb at approximately the distance to give total radiation values for midsummer. The bulbs of the thermometers were in open air ten inches above the table top where convection currents had full play. One bulb was dipped in wax, the other was not. At the end of one hour the waxed bulb thermometer read $51.5^{\circ}C.$, while the control read $50.0^{\circ}C.$ This $1.5^{\circ}C.$ heating effect was doubtless due in great part to decreasing the reflection of the one thermometer while maintaining the other as a good reflector. Heavy (7/1000 inch) cellulose film was now cemented directly to the bulb of the control thermometer which then gave the same temperature as the waxed one. When, however, a 1 by 1 by 2 cm. cellulose film box was slipped over this bulb, the temperature rose in fifteen

minutes to 65°C. or 15°C. above that of the waxed one.

In the second test the two thermometer bulbs were made good absorbers by painting them with lamp-black. They were then placed as above and stabilized in five minutes at 60°C. One thermometer was then dipped into the wax and again placed beside the other one. One hour later, the control (no wax) showed 62.2°, while the waxed one showed 62.5° or 3°C. warmer. The waxed bulb was then embedded in a lump of wax $\frac{1}{8}$ inch thick around the bulb. At the end of one, one and one-half, and two hours the waxed thermometer remained stabilized at 5°C. above the control.

used (figure 3B). The "waxed" leaf (waxed on both sides) then reached a maximum of 21°C., or 2.25°C. higher than on the preceding day. The "control" leaf (waxed heavily on the top side) reached a maximum of 12.5°C., which is also 2.25°C. higher than on the preceding day. The difference between the "waxed" and "control" leaves was therefore exactly the same for the two days, thus showing that the addition of the wax on the upper surface had little or no effect on the heating. This day was warmer and the sky clearer; the "waxed" leaf was dead and the "control" uninjured at the end of the day. In figure 3C is shown the last of this series. The "waxed" leaf was waxed only on

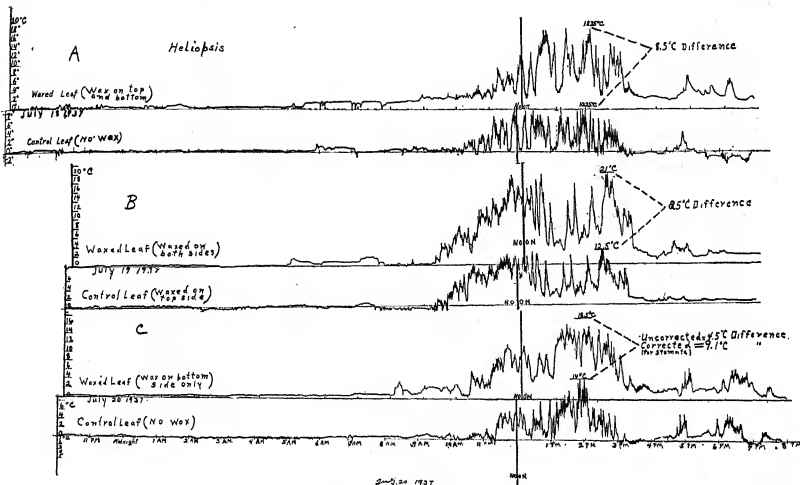


FIG. 3.—A. Curves for waxed and control leaves of *Heliopsis* on July 18 showing a difference in temperature of 8.5°C.—B. Similar curves for the following day: the waxed leaf is the same as on the preceding day, while the control is waxed heavily on the top; the difference in temperature is again 8.5°C.—C. Similar curves for July 20; the waxed leaf is waxed only on the lower side; the control is not waxed at all. The temperature difference uncorrected is 4.5°C., while after correction for stomata it is 9.1°C. See text for further details.

Even though the above data seem to preclude any chance that the waxing of leaves appreciably affects their heating characteristics, nevertheless a series of tests was made directly on the leaf on this point. The first of this series (July 18) is shown in figure 3A. The upper curve shows the temperature of the "waxed" leaf (waxed on both sides), while the lower shows the temperature of the "control" leaf (no wax at all). The "waxed" leaf reached a maximum of 18.75°C., while the "control" reached only 10.25°C., or 8.5°C. lower. The air temperature of this day was not high and the sky not clear, and neither leaf was injured by the test. On the following day, (July 19) the same two leaves were again

the lower side, and the "control" was not waxed at all. At the end of the day (a day similar to the preceding one) it was found that the "waxed" leaf had reached a maximum of 18.5°C., while the "control" reached 14°C. The stomata on the upper and lower sides were then counted, and it was found that 80 per cent were on the lower side. In other words, with 80 per cent of the stomata closed, the leaf heated to 18.5°C. If one neglects the greater reradiation which occurs at the higher temperature and calculates as a linear function the heating which would have occurred had all stomata been closed, the leaf would have reached 23.1°C., or 9.1°C. above the

"control." This is $0.6^{\circ}\text{C}.$ greater than on the two preceding days.

Maximum heating of non-transpiring leaves.—After the mass of preliminary work given above was complete, the fundamental problem of the maximum temperature to which a non-transpiring leaf would rise as a dark body was investigated. It is to be assumed in these studies, unless stated to the contrary, that the leaves were waxed on both sides and held as nearly normal to the sun during midday as was possible. Referring to figure 2A, we have the story for apple. In this test only the lower side of the leaf was waxed, since the upper had no stomata. The temperature of the leaf remained very low, as has already been noted, because of the orientation of the leaf. At 12:30 it was set normal to the sun,

neither was injured. However, on the succeeding day, which was clearer, the waxed leaf was entirely killed by overheating.

Figure 4A shows one of the most striking cases of this killing of leaves that we found. The waxed maple leaf used in the test for scattered junctions showed a rise of $27.75^{\circ}\text{C}.$ above air. The air temperature during the middle of the day was $29^{\circ}\text{C}.$, giving an actual leaf temperature of $56.75^{\circ}\text{C}.$ The leaf was entirely dead by evening. In figure 4B is shown the result with *Asclepias syriaca* with a maximum temperature for the waxed leaf of $21.8^{\circ}\text{C}.$ above air. The noonday air temperature was $32^{\circ}\text{C}.$, giving a maximum of $53.8^{\circ}\text{C}.$ There was little or no injury for this day, but on the following day, when the sun came out much more clearly, the leaf was cooked en-

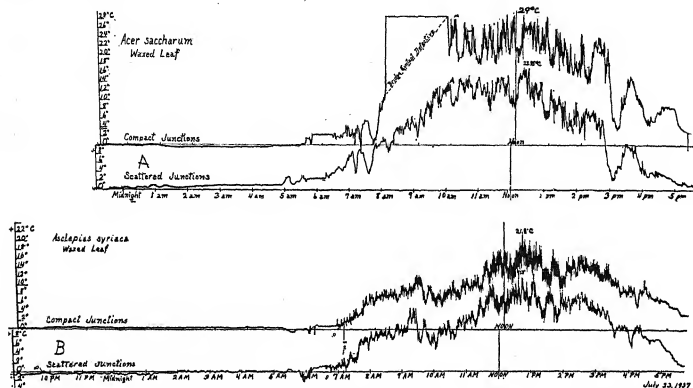


Fig. 4.—A. Temperature of waxed leaf of *Acer saccharum*. "Compact Junctions" curve for waxed leaf as given by junctions when arranged within a 6 mm. square area; "Scattered junctions" curve for same leaf at same time as given by junctions when scattered about the surface of leaf. The maximum temperature of $29^{\circ}\text{C}.$ above air for the first as compared to $27.75^{\circ}\text{C}.$ for the latter indicates no appreciable difference in sampling. The leaf was entirely dead by evening.—B. Similar tests of arrangement of junctions on *Asclepias syriaca*. In this case the compact arrangement gave a maximum of $21.8^{\circ}\text{C}.$ as compared to $23^{\circ}\text{C}.$, showing again no significant difference.

and the temperature rose to $18^{\circ}\text{C}.$ above air. Shortly after this the upper surface of the leaf was painted with wax to see if a further rise in temperature could be obtained, but without success. The control leaf, meantime, which was practically normal to the sun, had risen to about $8^{\circ}\text{C}.$ above air. It was likewise painted with wax on its upper surface at point "B," and this caused a temporary rise in temperature to $16^{\circ}\text{C}.$ (approximately $3^{\circ}\text{C}.$ below the melting point of the wax), but it quickly cooled to the level maintained prior to waxing. Neither the control nor the waxed leaf was injured by the test. In figure 2B is a similar story for *Chenopodium album*. Both leaves were normal to the sun at noontime, and the maximum temperatures reached were $18.5^{\circ}\text{C}.$ and $12.5^{\circ}\text{C}.$ for the waxed and control leaves, respectively, and

tirely white by 11:30 a.m. The control of course was uninjured.

In figure 5 is given the story for mullein, *Verbascum Thapsus*. The day preceding this test was cool, and the sun not out fully, so no injury occurred then. On the day for which the curves are shown, however, that part of the leaf which was waxed on both sides was entirely dead by 1 p.m. This test was of particular interest, not only because of the peculiar structure of the mullein leaf, which might cause it to give different results from the other more typical kinds of leaves, but also because the waxed and control were actually parts of the same leaf. The maximum temperature of $28.5^{\circ}\text{C}.$ plus $33^{\circ}\text{C}.$ for air, or $61.5^{\circ}\text{C}.$, was observed in a waxed region not more than two centimeters from the control portion, which

reached a temperature of only 52.5°C. and was entirely uninjured.

Figure 5 illustrates another important point concerning the cooling rate of waxed and normal leaves. The wide fluctuations in the curves are due mainly to alternate heating of the leaves in bright sunlight and cooling under passing clouds. It will be noted that the amplitude of the fluctuations is greater for the waxed leaf than for the control. Thus if we take the peak showing on both the waxed and control leaf curves just preceding noon, we find that the waxed one rose 21°C. in 14 minutes, while the control rose only 14°C. in the same time. Likewise on the fall, the waxed leaf dropped 26°C., while the control dropped 16°C. It seems hardly probable that the treatment with wax has any appreciable insulating effect. This is entirely reasonable, since if the wax rendered the leaf a poorer radiator, it would automatically reduce its absorption to the same extent, thus cancelling any effect. The straight line rise of the two curves just after noon, when the control rose 12°C. in four minutes, and the waxed rose 18°C. in the same time, is the most rapid major fluctuation in temperature recorded in these studies. The recorder is capable of showing a temperature change of 20°C. per minute. Minor temperature changes of 10° or less may take place as rapidly as the recorder can follow them.

Although it was realized at the time of the earlier work that the angle of incidence of light makes a great difference in the leaf temperature, and although leaves under comparison were always held at as nearly equal angles as possible, still the position of the leaves was less horizontal than was found in the present experiments to give the maximum heating. In the present work attached leaves were held nearly horizontal. In the earlier work the leaves stood upright in potometers with the blades sloping backward, or attached leaves were held in their natural positions. The author was trying to find a correlation between measured transpiration rates and the existing temperatures, and it was thought that as long as leaves under comparison were at equal angles to the sun the position used would bring out the correlation, if one existed. It did not occur to him at that time to try to find the maximum temperature of a leaf either as a non-transpiring or transpiring dark body, as is done in the present study. Figures 2 and 4 of this earlier work show that absorption did continue after the leaves were vaselined, but at greatly reduced rates. Arthur and Stewart (1933) also found that with high light intensity if both sides of a tobacco leaf were vaselined and the leaf placed in a cellophane bag, beads of moisture condensed on the inside of the bag, thereby showing that water escaped from the leaf

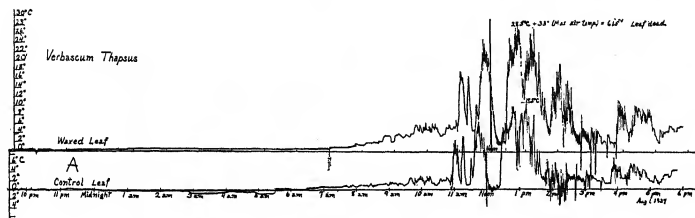


Fig. 5.—Temperature relations between waxed and control for *Verbascum Thapsus*. In this case both the waxed and control were parts of the same leaf, and the junctions were not more than two centimeters apart. The waxed parts were entirely dead by 1 p. m., while the control remained uninjured. Maximum leaf temperature 61.5°C. for the waxed and 19.5°C. for the control or a difference of 9°C. See text for discussion of heating and cooling rates.

Additional species, the leaves of which were killed by waxing and placing them normal to the sun, include *Althaea rosea*, *Forsythia viridissima*, *Phlox paniculata*, *Polygonum* sp., and *Syringa vulgaris*. No leaves so far tested were able to remain uninjured when thus treated. Below are given some preliminary data on *Opuntia* which seem to disagree with this general statement; but the cactus "pad" is not a leaf.

The temperatures here recorded for waxed leaves are higher than those previously reported by Clum (1926) for leaves with reduced transpiration, while the temperature of control leaves was 2° to 3°C. higher than the highest in the earlier work. Two factors may account for these discrepancies: the difference in angle at which the leaves were held to the sun, and the failure of vaseline to stop transpiration.

through the vaseline. Although no measurements of transpiration were made in the present study, the fact that the wax used does not materially affect the transmission, reflection, or reradiation of energy from the leaf points to the conclusion that the differences in temperature here observed must have been due to differences in transpiration. This viewpoint is also borne out strongly by the evidence of figures 3A, B, C, in which it was shown that waxing the upper surface of the leaf failed to make it heat up; while from the temperature of a leaf waxed on the lower side only, one can correct for the number of stomata and calculate very closely what the temperature of the leaf should have been if both sides had been waxed.

On first examination the data given in this paper might seem to differ greatly from those given by other workers, but closer study discloses that the

present paper differs from the previous work more in viewpoint than in data. Thus if we tabulate the differences in temperature between the transpiring and non-transpiring leaves we find no extreme values. In figure 1 and supplementary data, temperatures range from 7°C. below air to 4°C. above air. When one considers, however, that it took a continuous record of leaf-air temperature over a period of seven days to show this maximum range, it is obvious how unlikely it would have been found by anyone making discontinuous readings. It is therefore not surprising to find the 7°C. is 1.5°C. greater than the maximum cooling found by Clum for cabbage. In figures 2A, 2B, 3A, 3B, and 5, those curves for which values for both the control and waxed leaves are available, we find apparent cooling effects of 9.25°, 6.0°, 8.5°, 8.5°, and 9.0°C. These average about 3.0°C. higher than the maximum cooling reported by Clum for cabbage and are reasonable since the leaves in these curves were held normal to the sun instead of assuming the position characteristic of the plant, as in figure 1. For this same reason our maximum leaf temperatures should and do range with the highest reported by others. In studies on survival value only maximum temperatures are significant since they determine life or death during those crucial periods when plants growing in full sun are heated most.

One can take the data given in this paper and treat them in two different ways and arrive at the same results. First, let us take the control (transpiring) leaves, so the wax can play no part in the temperatures, and add to these maximum temperatures the cooling found by others. Clum (1926b), table 2, found, under the extreme conditions of an enclosed chamber, that a plant in dry soil was consistently 7.9°C. warmer over a three-hour period than another plant in the same chamber which was in wet soil. Since the two plants were in the same chamber and therefore exposed to the same conditions, it is safe to conclude that this difference must be due to the wet and dry soil, or, in other words, available water. If this 7.9°C. cooling effect is added to the temperatures of the control leaves in figures 1A, 1B, 2A, 2B, 3A, B, C, and figure 5, we find that a lethal temperature is reached only for figures 3B and 5. These are likewise the only ones in which the waxed leaves were killed.

In the second place, if one takes the data in figures 4A and 5 and subtracts from them this same 7.9°C. cooling, the leaves in neither of the tests would have reached a lethal temperature. This treatment of the data assumes that transpiration would not be less at higher temperatures, and that the two plants used in the curves were able to cool themselves as well as the *Fuchsia* which gave the 7.9°C.

In addition the problem can be approached in still another manner. If one takes data from various investigators (Smith (1909), 13° and 16°C.; Seely (1917), 14°C.; Clum (1926), 14° to 16°C.; Matthaei (1905), 17°C.) showing control leaf temperatures from 13° to 17°C. above air and adds it to a

reasonable mid-summer temperature of 35°C., the leaf must then be at a temperature of from 48° to 52°C. This is so close to lethal that there can be little doubt that transpiration is significant. In addition it may be necessary for us to modify our conceptions of transpiration and its significance in still another manner. If subsequent work shows that many species have their maximum cooling at the time of wilting, as shown above for *Heliopsis* in figure 1, we must then consider transpiration as affecting leaf temperatures both directly by physical evaporation and indirectly by changing the angle of the leaf to the sun. The total transpiration temperature effect would then be in the range of 15° to 20°C., since changing the angle of the leaf may affect the temperature as much as 11°C. (fig. 2A).

Leaf, stem, and flower temperatures.—Some tests were also made on other plant parts. Thus in figures 6A and 6B are shown curves for flowers, stems and leaves of *Althaea rosea*. The leaf temperature curve is the usual type, being above air for most of the time but passing below it when the direct rays of the sun ceased to strike the leaf in the afternoon. The stem temperature was taken by clamping the thermopile on the south side of the stem in the sun, except for a 15-minute period at 10 a.m. when the junctions were clamped on the back side of the same stem. The back side was only slightly above air temperature at the time the front side was 7°C. above air. The maximum leaf temperature and the maximum stem temperature for this particular day were the same, 11.5°C. above air, but the stem temperature never went below air. Simultaneous tests on flowers showed that hollyhock petals give about the same story as leaves. In figure 6B are some additional data on petals. In this test red and white petals were waxed and held normal to the sun. Both curves reached a maximum of about 14°C. above air, and neither was injured by the treatment. The sudden drop on the white-flower curve is due to the petal no longer being in direct sunlight. The day following this test both petals were left normal to the sun. The day was much warmer, and the sun brighter, and both petals were dead by evening. These preliminary data would indicate that petals and leaves are much alike in their temperature relations.

Temperatures of Opuntia.—Preliminary tests have likewise been made on *Opuntia* (Burbank Spineless). In these tests the cactus pads were treated as leaves. The potted plants were placed so that one pad was merely oriented normal to the sun as a control (fig. 7B), a second was waxed and placed normal to the sun (fig. 7A), while the third was painted black (fig. 7C). The two days, August 2 and 3, on which these tests were run, were very clear and the hottest recorded for the season (34°–40°C.). Any non-transpiring leaves previously studied would have been quickly killed. The cactus was, however, uninjured. Reference to figures 7A and 7B will show that to all intents and purposes the waxed and control pads were alike, the maximum temperature of the first being

12°C., while that of the second was 12.75°C. It should be pointed out that these temperatures were on the "bottom" sides of the pads (away from the sun) as in the case of the leaves. The pad that was blacked was slightly less than 2°C. higher than the control pad. In the summary in figure 7A it will be seen that the temperature values for the two days were practically identical. It was thought that a measurement of the temperature of the upper surfaces of the pads would probably be much more nearly analogous to those for the thin leaves studied earlier. For this reason, on the third day the test was set up with one set of junctions on "top" of a

same manner as above in figures 7D and 7E, curves were obtained with maximum temperatures for the "top" and "bottom" of 34°C. and 24°C., respectively, this differential of 10°C. being the same as for curves D and E. The natural pad (waxed) gave a maximum of 25°C. for this day. A waxed geranium (*Pelargonium zonale*) on the same day gave a maximum temperature of 30°C., only 4°C. below that of the artificial pad. Just what these data mean and just why a thick structure like a cactus pad can remain much cooler after hours of exposure to full sun than thin structures like leaves, even with transmissions and reflections similar, we do not know. It

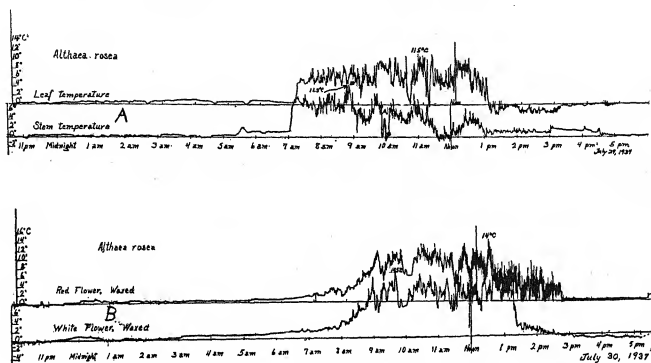


FIG. 6.—A. Curves for leaf and stem temperature of *Althaea rosea*. Maximum for leaf 11.5°C., for stem the same, 11.5°C. Simultaneous curves for flowers were similar to that for the leaf.—B. Curves for a white and for a red flower of the same species. These petals were waxed and held normal to the sun. Neither was injured the first day due to low radiation intensity, but both were killed the same as leaves the second day. The maximum temperatures the first day were 14.5° and 14°C., indicating little difference in heating due to difference in flower color.

pad, and the other set on the "bottom," which gave the curves shown in figures 7D and 7E. The "top" of the pad ran approximately 10°C. higher than the "bottom," but, as will be noted, the "lower" side of the pad gave nearly the same maximum as the day before, 12.5°C. The maximum on the "top" side was 22.5°C. which is very low indeed when one considers that the thin, waxed leaves of such plants as maple, milkweed, lambs quarter, etc., showed temperatures as high as 28° and 29°C. for radiation intensities much less than that obtaining during the three days of the cactus tests. Needless to say, the cactus exhibited no ill effects from the treatment. Some further preliminary work has been done by making up an "artificial pad" of clear photographic films (twenty layers with wooden separators between the layers) and filling it with dilute india ink to give just the total radiation transmission of the natural pad, about 3 per cent, and waxed so as to give approximately the reflectivity of the waxed natural pad, 29 per cent. When this "pad" was measured in the

would seem from the fact that a waxed geranium leaf rose to within 4°C. of the temperature of the artificial pad that the normal cactus pad should at least do as well. It is perhaps best for the present merely to take the data as indicating lines of investigation and wait until much more has been done under better climatic conditions before drawing conclusions.

Sky radiation and leaf temperature.—Curtis (1936b) has recently called attention to the interesting possibility that radiation loss to a clear sky may explain the fact that leaves are often at a lower temperature than the surrounding air. The loss of energy from the surface of the earth to the sky is of course fundamental to the heat balance of the earth. Since the earth as a whole is neither warming up nor cooling off, this loss, to space must be just balancing the energy receipt from space—primarily from the sun.

Above have been given curves for many leaves showing their temperatures for both day and night. If, then, leaves are cooled below air by their radiation to the sky, one must of necessity find evidences

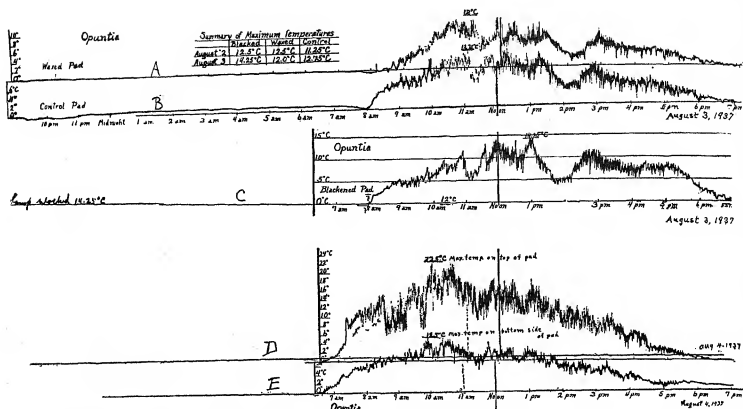


Fig. 7.—A. Curve for a waxed pad of *Opuntia* (Burbank Spineless) with a maximum temperature of 12.0°C. above air.—B. Similar curve for a normal pad with a temperature of 12.75°C.—C. A similar curve for a normal pad painted with lamp black and showing a maximum temperature of 14.25°C. In these three curves the pads were treated the same as leaves; the junctions were in contact with the "bottom" side of the pad, away from the sun, and the pads were held normal to noonday sun.—D. and E. Curves for the "top" and "bottom" sides of the same pad; maximum temperature for D, 22.5°C., for E, 12.5°C. See text for further descriptions.

of the phenomena here, since these curves represent the ecological conditions of cloud and clear present in this region. Referring to these curves, it will be recalled that in most of these tests two leaves were used, one being waxed to eliminate loss of water and the other being unwaxed as a control. If one finds the control leaf at a temperature lower than the air, it does not follow that radiation to the sky is responsible, since at the same time transpiration may be going on. If, on the other hand, one finds the waxed leaf cooler than the air, it would be strong evidence that energy loss to the sky might be responsible. Examination of all curves given above will disclose, however, that, as a rule, the control leaf is at or near air temperature during the night. The waxed leaf, on the other hand, is almost invariably above air temperature, not uncommonly as much as 1°C. It might be argued, however, that the curves are for cloudy nights. Examination of two sets of curves for two clear nights, figure 4A for maple and figure 5 for mullein, will show that the conditions just mentioned for the general relationship are likewise true for the very clear nights. It will be recalled that the rates of temperature rise and temperature fall of the waxed leaf show it to be at least as good a radiator as the control, and in spite of this the leaf remains slightly warmer than air most of the time. It is entirely natural and to be expected that during some nights a dip or rise of either the waxed or the control or both may occur, since cool or warm convection currents of air may pass by, or there may even be a temperature inversion (especially on clear nights), as

pointed out by Shreve (1912) or more recently by Turnage (1937). The latter reports that for radiation near the desert laboratory these inversions were usually around 9°F.; those of 15°F. were not unusual; and even one of 23°F. was found. Inversions of this magnitude are not common in southern New England but minor ones do occur as evidenced by the common summer fogs. Cooling due to radiation loss to the sky, on the other hand, would be constant so long as the sky was clear, and when clouds appear, one should find the curves fluctuating up and down.

It would seem from these smooth curves for the night period that radiation loss to the sky could play only a very minor part. It might be well, therefore, to examine meteorological data to see if these leaf temperature data seem logical. At any point on the surface of the earth the energy reaching that point is the summation of two components, the direct rays of the solar beam and the diffused rays of the sky radiation. Perhaps few of us in physiology realize the magnitude of this sky radiation. According to Kimball (1923) sky radiation constituted 13.5 per cent of the total radiation at high noon on April 2, 1923, at Washington, D. C., or an equivalent of .175 gram cal. min. cm². Five hours earlier, at 7 a.m., this value was .09 gram cal., while four hours later at 4 p.m. the sky radiation was .145 gram cal. or only a little less than at noon. This seemingly paradoxical relation of sky radiation at noon and in the evening is due to the fact that the curve for sky radiation comes up to almost full value within a short time after sunrise, remains flat-topped for the middle of

the day, when the solar beam is most strongly absorbed, and sinks shortly before sunset. Thus from about 10 to 40 per cent of the energy reaching the earth may be from the sky. The season of the year has some effect on the percentage; for instance, in Washington, D. C., Kimball (1927) found for solar zenith angle of 78.8° , or 45 minutes before sundown, that the sky radiation amounted to the following percentages of total radiation: for winter 37 per cent, for spring 40 per cent, for summer 40 per cent. Mount Wilson gave 38 per cent sky radiation for the same angle.

It is obvious from these data that one must consider the sky not merely as a source of energy loss, but also as a source of energy gain. In other words, the earth receives energy from the sky during the day just as surely as it does from the sun. That point in the evening at which the incoming energy from sky radiation just equals the loss of energy to the sky is the point of zero transfer. This is then followed by the night period during which there is a negative energy relation from the sky, and the surface of the earth cools by radiation to the sky. The exact time at which this reversal of energy relation between the sky and the earth occurs varies with location, atmospheric conditions, etc. In view of this, determinations were made with a Shirley radiometer to determine when the reversal occurred. This instrument consists essentially of a grid of thermal junctions. Those junctions on the top of the grid are blackened to render them good absorbers, while those on the bottom are whitened to render them good reflectors. When the cell is held with the black junctions towards a source of radiation, the black junctions warm up, while the white junctions tend to reflect the radiation hitting them from the back, thus building up a temperature difference which is expressed as millivolts, or gram calories. We have, then, in this instrument, a device for measuring radiation from the sky or sun on the black junctions, while simultaneously the earth radiation fails to exert its full force, since the white junctions reflect most of it. This instrument is not suitable for quantitative determinations of gram calory values of earth and sky radiation, but it does offer a simple method of showing which of these is greater. It is used merely in this plus or minus sense in this work. The device is enclosed in a glass container which eliminates air currents.

This radiometer was connected to one of the recorders and run continuously for four days (Sept. 7-10). The first day and night were entirely clear; the second was a clear day and a night with large "white" clouds; the third day and night were clear and the fourth overcast. During the daytime the instrument recorded total radiation reaching the instrument from sun and sky. At night it should measure the differential in energy exchange between the white junctions facing the earth and the black junctions facing the sky. On the evening of the first day tests were begun just 10 minutes before the sun set. The cell was shaded from the glancing rays of

the sun, and the instrument allowed to come to balance. The instrument would then be measuring the differential between energy income and outgo to the sky. This differential was sufficient to give a positive deflection of 8 mm.² on the recorder drum. The zero was determined by setting a black box over the cell to shield it from all sky light. At ten-minute intervals thereafter this box was put over the cell and then removed. The deflection on the record decreased to 5 mm. just as the sun disk disappeared; ten minutes later it had dropped to about 3 mm., and ten minutes after that the deflection of the null galvanometer just indicated a plus value, but it was not large enough to activate the recorder. It would seem, therefore, from these results, that the zero exchange of energy occurred by 20 minutes after sundown. While there are no direct conversion factors for twilight foot-candle values to gram calories, the general magnitudes of the values in this series are quite similar to those in the series of foot-candle values given by Humphreys (1927, page 550) for twilight, measured photometrically. This shows 33 foot-candles for sundown, 30 fc. four minutes later, 15 fc. eight minutes later, 7.4 fc. twelve minutes later, 3.1 fc. for 16 minutes later, and 1.1 fc. for twenty minutes after sundown. It should be pointed out that the foot-candle value can never become negative, but that the gram caloric value does, just as soon as the energy loss exceeds the energy gain.

Three hours after sundown (8:30 p.m.) the tests were begun again to determine, if possible, the magnitude of the energy loss to the sky. The record was running at that time as a straight line with very minor "jiggles," which showed that everything was working with the sensitivity desired. The cell was exposed to the clear sky. The leads from the cell were shorted at the recorder to find the amount of negative deflection given by the sky. The recorder was not even activated by this, showing that there was no appreciable temperature difference between the black and the white junctions. One of the operators then placed his hand above the cell while the other watched the recorder. The recorder was immediately activated and gave a plus deflection of 5 mm. for the heat received from the hand. The hand was removed, and the instrument went back to zero, so that again shorting the leads failed to register a difference between the black and the white junctions. The black box was placed over the cell, and the recorder failed to register. A pan of ice cubes was next placed on top of the cell, and the recorder showed 2 mm. minus for the energy absorbed by the ice. These results would seem to indicate that there is no loss of energy to the sky, which is impossible. Next the cell was laid on a piece of tin foil with the black junctions up. A 2 mm. negative reading was found. It was obvious, then, that negative readings had failed to show up before. The cell was receiving earth radiation from below sufficiently rapidly to prevent

² At noon the deflection for the total radiation from sun and sky on a horizontal surface was 120 mm.

the development of any appreciable temperature difference between the black and the white junctions. A radiometer cell is so constructed as to maintain the greatest possible temperature difference between junctions. In this respect the unit is doubtless a far poorer conductor of heat between junctions than is a leaf full of water in conducting heat from the lower surface, where it receives earth radiation, to the upper surface, where it is radiating that heat to the sky.

This would seem to be the fundamental reason why the waxed leaves failed to show temperatures below that of air. It would also indicate how only by the greatest care could one apply cooling or warming effects, such as Curtis got in his black boxes, to anything in nature. It is almost impossible in nature for any object to be so located that it does not intercept radiation from the earth, and since it does receive such radiation, it should not go below the temperature of the air, unless those bodies radiating to it are likewise cooler than the air.

On the following evening the same series of tests was made and similar results were found. The black box again made the cell read zero. The pan of ice cubes (these readings were made just at sundown again) reduced the reading somewhat, but it still showed a plus 3 mm.; when the black box was placed over the cell, the reading became a minus 2 mm. as on the evening before. Another very striking demonstration of the potency of radiation on the readings was found when a large, white cardboard was held two feet above the cell, shielding it from the sky. A drop in the positive reading was expected similar to that given by the box. But instead the deflection increased, and the recorder moved upward very slightly. Further testing showed that the earth radiation being reflected back from the white cardboard was causing the reading. Tests with tin foil gave larger readings, while laying the cell with the white junctions down on one piece of the foil and pushing another down on top gave the usual zero reading. It would seem, therefore, that using a shield, as was done by Curtis, may actually increase the temperature reading, not because the loss to the sky is cut off, but because the shield reflects back the earth radiation.

SUMMARY

Tests during the period from June 15 to September 1 on twenty species of woody and herbaceous plants were made by means of thermopiles and recording potentiometers in a reexamination of the problem of leaf temperatures and their significance in the economy of the plant. One such test on *Heliopsis*, extending from July 2 to 9, inclusive, showed for this period a maximum cooling below air of 7°C. and a maximum heating above air of 4°C. It seemed highly doubtful whether this small variation was significant with ordinary conditions. The question which did seem important was, "To what temperature will a non-transpiring leaf heat when exposed normal to the rays of the sun?" Vaseline failed to prevent trans-

piration in leaves. Waxed leaves with a mixture of beeswax and rosin prevented transpiration and can be used, if care is taken, without damaging the leaves. Determinations have shown that leaves thus treated have a decreased relative transmission of 3.7 per cent and an increased relative reflection of 4.3, which indicates that theoretically a waxed leaf should heat up less than a normal leaf, if transmission and reflection were the sole factors involved. Some plants may be protected from overheating by wilting. *Heliopsis*, for instance, showed the maximum cooling of 7°C. at the time of maximum wilting of leaves. A non-transpiring (waxed) leaf of apple, edgewise to the sun, was cooler than a control leaf normal to the sun, but the former quickly rose 11°C. higher when placed normal.

All species tested have been killed when waxed and held normal to the sun, except on cloudy days when radiation intensity was low. Leaf temperatures of 58°C. to 60°C. are common in waxed leaves on days with normal radiation intensities. Control leaves normal to the sun on the same days were as much as 19.5°C. above air. The differential between the waxed and control leaves was from 6° to 9°C., indicating a cooling effect of this amount. No conclusions are made as to the quantitative heating effects of different species. Further tests in a region of more uniform radiation conditions must be made. There seems to be no doubt, however, that transpiration is necessary at times to prevent leaves or leaf parts normal to the sun from being injured. Preliminary tests on leaves, stems, and flowers indicate no marked difference in temperature relation of parts.

Preliminary tests on *Opuntia* have shown no significant temperature difference between control, waxed, and blacked pads. The cactus pad when held normal does not heat up as much as would be expected from its transmission and reflection values. An artificial pad made to simulate the cactus as to size and optical properties went to 34°C. above air at the same time the cactus pad rose to 25°C. A waxed geranium leaf rose to 30°C. under the same conditions. The cactus requires much additional study before conclusions can be made.

An examination of the curves in this paper indicates that loss of energy to the sky does not explain the fact that leaves are often below air temperature. Meteorological data indicate that this must be true for the daylight period, since energy receipt from the sky then exceeds the energy loss to the sky. Likewise at night leaves are seldom, if ever, below air temperature due to loss to the sky, since they intercept outgoing earth radiation which will keep them at the temperature of surrounding objects. These conclusions were verified by readings of a total radiation cell.

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THE SIMULTANEOUS MOVEMENT OF CARBOHYDRATES AND FLUORESCIN IN OPPOSITE DIRECTIONS

IN THE PHLOEM¹

Edward M. Palmquist

THE HYPOTHESIS proposed by Münch in 1926 to account for the mechanism of solute transport in plants was at first rather widely accepted. Possibly the immediate acceptance of this hypothesis, which proposed a mass flow of materials through sieve tubes, resulted from the fact that it is based on sound physical principles, or that such a mass flow can be demonstrated with a simple apparatus. More recently, however, the theory has been questioned, for, in spite of its sound physics and ease of demonstration, the processes involved seem to be inconsistent with certain observations made on plants. The subsequent similar theory advanced by Crafts (1932), who merely extended the possible path of movement to the entire cross-section of the phloem, may be criticized on the same grounds.

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The writer takes this opportunity to express his appreciation to Professor O. F. Curtis for his stimulating interest and many helpful suggestions during the course of the work.

Curtis (1935) has recently evaluated both these theories and has pointed out that, among other weaknesses, neither of them allows for the simultaneous movement of different solutes in opposite directions in the phloem. Indications that this actually takes place in plants have been recorded by several investigators. Caldwell (1934) has reported that both the rate and direction of the movement of certain viruses were independent of the movement of carbohydrates. The viruses studied, however, moved rather slowly and possibly in tissues other than the phloem. Samuel (1934) likewise indicated that the virus in his investigation moved at times in a direction opposite to that of carbohydrates. This virus moved rapidly, and apparently in the phloem tissue. Concerning the movement of materials in uninfected plants, many ringing experiments might be cited in which the movement of nitrogen and sugar seems to occur in opposite directions. The results reported by Curtis (1937) point strongly to this conclusion. The object

of Curtis's work was to determine whether or not minerals could be carried by the phloem from the roots to the leaves. Using shoots of *Salix*, he found that over a three-day period only an extremely insignificant quantity of nitrogen moved from the roots past a ring to the upper part of the stem, while in similar shoots not ringed, but with a section of xylem removed, the movement of nitrogen approached closely that in intact plants. Because of the small number of shoots used, these results were not clearly significant when treated statistically. It is reasonable to believe that during the period of this upward movement of minerals through the phloem, carbohydrates were moving downward into the roots through the same tissue, but no specific determinations were made, and thus, although the data are strongly indicative, simultaneous movement in opposite directions was not demonstrated conclusively.

Very recently, in fact since the inception of the present work, Mason, Maskell, and Phillis (1936) have reported work in which this question was tested specifically. Their results indicated that nitrogen and sugar may move in opposite directions at the same time in the phloem of cotton plants. All of their plants were ringed in the internode above the cotyledonary node. Black paper bags were placed over all leaves below the eighth internode from the apex. The portion of the plant extending from this internode down to the ring was termed the "basal region," and the portion above it, with leaves exposed, was called the "apical region." One group of these plants then received an additional ring between the basal and apical regions—that is, in the eighth internode. The plants in this group were called the "ringed" plants, while the others which had only the original ring at the base were designated as "normal" plants.

The apical region of normal plants gained nitrogen without exception. In the ringed plants, some of the apical regions gained nitrogen while others remained unchanged. Where gains occurred in the ringed plants, they were not as great as those in the normal plants. This would seem to indicate that at least some nitrogen moved up through the phloem of the normal plants from the basal region to the apical region. The movement of nitrogen through the xylem past a ring between those regions in some of the ringed plants, however, indicates that the nitrogen may have moved in the xylem in the normal plants. The basal portion of all plants lost nitrogen; the losses in the normal plants, however, were greater than those in the ringed plants. This likewise indicates movement of nitrogen upward through the phloem, for when a ring of phloem was removed the movement was retarded. Since some nitrogen was lost in the ringed plants, the proof is by no means conclusive that all of it moved in the phloem of normal plants.

Having thus fairly well established that nitrogen moved up through the phloem, Mason, Maskell, and Phillis examined the plants for indications of carbo-

hydrate movement. Dry weights of the apical regions were determined and, surprisingly, no significant differences were found, both ringed and normal plants having increased in dry weight in the apical region. The writers point out, however, that there was indication of downward movement of carbohydrates in the phloem, for the basal regions of the ringed plants decreased in dry weight more than did those of normal plants, and when the dry weights of the apical regions are expressed as percentage of the dry weight of the combined apical and basal regions, it is shown that the ringed plants were relatively heavier in the apical region. (It might be pointed out, however, that in one of their two groups of plants the actual dry weights of the basal regions of ringed plants exceeded those of the normal plants in three of the six collections.)

The writers also compared the apparent carbohydrate content of the apical regions of the two groups of plants. The apparent carbohydrate was calculated by subtracting 5.7 times the weight of nitrogen from the dry weight. This value for the apical regions of ringed plants is significantly greater than that for the normal plants. If this calculated value can be taken as an accurate estimation of the actual carbohydrate, there can be little doubt that downward movement of sugars occurred in the phloem. The accuracy of the calculated apparent carbohydrate, however, is questionable; it is based upon the assumption that the newly acquired increment of nitrogen is combined with other elements in the same proportion as the nitrogen present in the apical region before the experiment began. If the proportion of nitrogen in the newly acquired nitrogen compounds were greater than that of the original nitrogen compounds, the factor 5.7 would be too high for the normal plants, and the difference between the two groups would lose in significance. In spite of this possible criticism, however, it seems the writers were justified in stating that carbohydrates moved downward through the phloem. Having thus demonstrated that nitrogen moved upward and carbohydrates moved downward in their plants, they concluded that those two solutes may move simultaneously in opposite directions in the phloem.

This conclusion is not entirely justified, for, even had they demonstrated conclusively that nitrogen moved up and carbohydrates down in the phloem of their plants, their collections were taken at intervals of at least two days, and it is possible that the two solutes moved in opposite directions at alternate periods of time within the two-day period. Fischer (1936) likewise has concluded that nitrogen and carbohydrates can move simultaneously in opposite directions in the phloem. The data which best support his conclusion were taken from four plants of *Pelargonium*. He darkened these to lower the carbohydrate and nitrogen content of the leaves. One half of the lamina of three leaves on each plant was removed and held for labor analysis. The remaining halves, still attached to the plant, were covered with

tin foil, and the plants exposed to daylight for a period of from seven to ten hours. The dry weight and total nitrogen content of the two halves of each leaf were then compared. In six of the twelve leaves from these four plants, there was an increase in dry weight and a decrease in nitrogen during the ten-hour period. Of the remaining six leaves, three lost in both nitrogen and dry weight, two gained in nitrogen and dry weight, and one lost in dry weight and gained in nitrogen. Considering that the analyses were made on individual half leaves, and that only half of the data support the conclusion, this work is hardly convincing. Fischer reports other data which are slightly more consistent, but these involved periods of several days, and therefore may be criticized on the same basis as those of Mason, Maskell and Phillips—that is, nitrogen and sugar may conceivably move in opposite directions at alternate periods of time. The writer believes that the work reported in the following pages meets this objection, in that one of the solutes concerned was followed visually at short intervals of time.

METHODS AND MATERIALS.—The solutes investigated were carbohydrates and the water soluble sodium salt of fluorescein, known as uranin. Fluorescein was selected because it is easily detected in extremely minute quantities in plants, and unlike eosin, phloxin, and erythrosin, the halogen substituted fluorescein compounds, it seems to have no harmful effect on the phloem. An apparatus similar to that described by Schumacher (1933) was used to detect the presence of fluorescein. An intense source of light was supplied by a carbon-arc lamp. The carbon sticks in the lamp were 10 millimeters in diameter, and the arc was generated with direct current at 110 volts. The light from this arc was condensed by a lens and passed through a one-centimeter layer of 10 per cent copper sulphate solution which served to remove a large fraction of the infra-red and longer red rays. This filtered light then passed successively through the two blue Corning Glass filters, Nos. 554 and 585, which were used in place of Schumacher's Uvetglas (U-Gl. Schott u Gen. Jena). The light reflected by the microscope mirror to the object was therefore almost entirely within the blue-violet range of the spectrum. A small disk of Eastman Kodak (G-15) gelatin filter was placed between the ocular lenses to serve the purpose of the ocular cap of Euphos glass used by Schumacher. This filter is transparent to only the yellow-red range of light, therefore the field of the microscope is barely discernible as a dull grey disk when no fluorescent body is under the objective. Fluorescein solution when exposed to blue-violet light emits an intense greenish yellow light; this fluorescent light, having a wave length of .542 microns, passes through the ocular filter. With this apparatus, an object containing fluorescein stands out as a vivid greenish yellow body in a dark background, while one which does not fluoresce appears, if at all, as a shadow.

Unfortunately for this line of investigation, many plants possess inherent fluorescent compounds, some of which resemble fluorescein very closely. Mature wood, in all the varieties of plants examined, fluoresces in various shades of yellow. Portions of the bark of these plants likewise fluoresce various colors from red to yellow. The cutin of the herbaceous plants examined produces a greenish yellow fluorescence very similar to that of fluorescein. The choice of material with which to work is therefore restricted. Woody plants in general have too much natural fluorescence which might be mistaken for that of fluorescein. The most satisfactory plant of those tried in the present work was the common red kidney bean, *Phaseolus vulgaris*. A cross-section of the stem of this plant appears mostly red under the fluorescence microscope because of the abundance of chlorenchyma cells. The section is outlined by a thin line of greenish yellow light produced by the cuticle. The phloem is without color, while the xylem is a grayish yellow which does not resemble the greenish yellow of fluorescein.

Fluorescein was introduced into the plant by immersing the terminal leaflet of a compound leaf half way in a 0.1 per cent aqueous solution of the sodium salt (Fluorescein Merck Na.-water soluble). The distal half of the midrib of this leaflet was scraped slightly on the dorsal side, just before immersing, to remove the cuticle. Schumacher applied fluorescein in a similar concentration to his *Pelargonium* plants, but added five per cent gelatin and placed droplets of this semi-solid solution over the slightly scraped veins of leaves. He found that the fluorescein entered the veins of the leaves, and in about an hour after the application it had traveled in the sieve tubes down through the petiole of the leaf into the stem. Fluorescein likewise enters the immersed terminal leaflets of bean plants and moves at a similar rate downward through the phloem into the stem. It also moves up into the two lateral leaflets of the leaf to which it is applied. One hour after the application, a cross-section of the petiole of the treated leaf appears under the fluorescence microscope exactly as that of an untreated one, with the exception of the phloem, which in the treated leaf is a brilliant greenish yellow, indicating, as Schumacher has pointed out, that fluorescein travels in the phloem. This is further substantiated by scalding a portion of the petiole. In well watered plants fluorescein does not pass a section of a petiole or rachis that has been killed by scalding. In plants that are deficient in water, however, fluorescein may pass scalded regions, but not in the phloem. When a terminal leaflet of a water deficient plant is immersed in fluorescein solution, water is absorbed and flows downward through the xylem of the leaf into the stem, carrying fluorescein with it. Because fluorescein does not pass a scalded region of a petiole in a well watered plant and because microscopic examination shortly after treatment shows fluorescein in the phloem only, it is evident that the normal path of movement is the phloem.

Rhodes (1937) has indicated that fluorescein moves more rapidly in the xylem than in the phloem of the several plants he examined, but his findings obviously do not apply to bean plants.

EXPERIMENTAL DATA.—The two lateral leaflets of most compound leaves of bean plants appear very closely congruent. The dry weights of the two leaflets are likewise very similar. In a series of leaves tested, the average difference in dry weight between the two leaflets picked at the same time was less than one per cent. An overnight loss in carbohydrates from one of these leaflets can be demonstrated easily by removing one of the pair in the afternoon and the other the following morning. In a series of leaves so treated, the average dry weight of the leaflets removed in the morning was about eight per cent less than that of the leaflets removed in the preceding afternoon. To determine whether or not this loss of carbohydrate would occur while fluorescein was entering, the following experiment was performed. Ten potted bean plants were removed from the greenhouse

TABLE 2. *A comparison of the dry weights of paired lateral leaflets from fluorescein treated leaves before and after a 15 hour period in darkness.*

Dry weight of leaflet A in milligrams	Dry weight of leaflet B in milligrams	Apparent loss as percentage of A
46.4	47.8	-3.02
42.7	44.3	-3.75
52.0	45.1	13.27
64.4	54.4	15.53
47.6	43.6	8.40
59.3	51.2	13.66
34.0	34.2	-0.59
43.3	39.1	9.70
53.9	46.3	14.10
43.7	41.7	4.58
Av. 48.73	44.77	8.13
Odds: 132:1		

TABLE 1. *A comparison of the dry weights of paired lateral leaflets from fluorescein treated leaves before and after a 15 hour period in darkness.*

Dry weight of leaflet A in milligrams	Dry weight of leaflet B in milligrams	Apparent loss as percentage of A
79.6	71.4	10.30
73.0	64.1	12.19
59.0	61.0	-3.39
57.4	52.8	8.01
53.7	44.4	17.32
41.8	35.3	15.55
55.9	54.8	1.97
24.8	22.7	8.47
49.7	46.3	6.84
47.8	41.6	12.97
Av. 54.27	49.44	8.90

Odds: over 666:1^a

^a H. H. Love's (1924) modification of Student's Table was used in calculating the significance of data.

to a dark room at 5:00 p.m. and watered. A symmetrical compound leaf was chosen on each plant, and a lateral leaflet was removed from each of them. These detached lateral leaflets were placed in paper envelopes marked A and laid on their respective pots. The terminal leaflets of the same leaves were then scraped very lightly on the dorsal surface and immersed about half way in fluorescein solution. The following morning at eight o'clock the remaining lateral leaflets were removed and cross-sections of their petiolules were examined for the presence of fluorescein. Without exception fluorescein had moved out of the terminal leaflets down into the petioles and out into the lateral leaflets. The amount which had moved was sufficient to cause the veins of the lateral leaflets to appear a bright greenish yellow

when examined in blue light without the aid of the microscope. These leaflets were then placed in envelopes marked B, and, with those removed the previous evening, were placed in the vacuum oven to dry for three days. They were then removed, cooled in a desiccator, and weighed. The dry weights in milligrams and the apparent loss after a 15-hour period in darkness expressed as percentage of the dry weight of leaflet A may be found in table 1.

This experiment was repeated twice. Fluorescein was invariably found in the attached leaflets. The dry weights of the lateral leaflets detached at the beginning of the experiment (A) and at the end (B) and the apparent losses may be found in tables 2 and 3.

TABLE 3. *A comparison of the dry weights of paired lateral leaflets from fluorescein treated leaves before and after a 15 hour period in darkness.*

Dry weight of leaflet A in milligrams	Dry weight of leaflet B in milligrams	Apparent loss as percentage of A
43.0	38.5	10.47
73.3	73.3	0.00
33.0	29.1	11.82
31.7	31.0	2.21
50.1	45.3	9.58
40.4	32.4	19.80
34.7	29.0	16.43
63.7	59.7	6.28
53.7	48.5	9.68
48.0	42.1	12.29
Av. 47.16	42.89	9.05
Odds: over 4999:1		

These data indicate clearly that carbohydrates have moved out of the attached lateral leaflets during the experiment. Previous experiments have shown that

the path of this movement is the phloem (Curtis, 1925). Fluorescein likewise moves in the phloem, and since there is but one vascular bundle in the petiole of a bean leaflet, the two solutes moved in the phloem of that bundle in opposite directions during the period of the experiment. Cross-sections of petioles taken at random intervals of time from leaves treated as those in the experiment invariably revealed the presence of fluorescein in the phloem; therefore, the outward movement of carbohydrates must have occurred while fluorescein was moving into the leaflets.

To test the matter further, experiments were planned to determine whether or not carbohydrates could move into leaflets while fluorescein was moving out. Mason, Maskell, and Phillis (1936) have reported that carbohydrates move into a darkened, previously starved leaf when a ring of phloem is removed from the stem just below the leaf. They found that sufficient carbohydrates had moved into the leaf in about three days to form a quantity of starch large enough to be detected with iodine. This suggested the possibility of demonstrating the movement of carbohydrates into similarly treated bean leaflets. An envelope, made of black paper and coated on the outside with aluminum paint, was placed over the terminal leaflet of each of fifteen leaves of potted bean plants. After three days, five of these terminal leaflets were collected and tested with iodine for the presence of starch. No starch was found in any of them. A portion of the petiole about two centimeters in length was then scalded on each of the remaining leaves. This was accomplished by wrapping a small piece of cotton about the petiole and percolating about twenty cubic centimeters of boiling water through it from a wash bottle. Five of the darkened terminal leaflets were collected and tested with iodine on the day following this treatment, and a trace of starch was found in the main veins of each of them. The remaining five leaflets were collected two days after the petioles were scalded, and in these all the veins showed a distinct starch test. Obviously the isolation of the leaf from the rest of the plant by scalding the petiole had resulted in a movement of sugars from the lateral leaflets, which were exposed to light, into the previously starved terminal leaflet.

An experiment was then planned to determine whether or not this movement of sugars into the terminal leaflet would occur while fluorescein was moving out. The terminal leaflets of fifteen leaves were darkened with the aluminum-painted envelopes for three days. Five of these were then tested and failed to show the slightest trace of starch (fig. 2). The remaining ten were treated as illustrated in figure 1. Dark chambers were prepared by covering test tubes successively with black lacquer and aluminum paint. These were then filled about two-thirds full of fluorescein solution. The upper half of the midrib of each terminal leaflet was scraped slightly on the lower surface, and the entire leaflet was placed in a test tube. The rachis was then packed with cotton. Thus the proximal unscraped portion of the leaflet was in

contact with air in the darkened test tube, while the scraped portion was immersed in fluorescein solution. The petioles were then wrapped with cotton and scalded.

Two days later the treated leaves were removed from the plants and the petioles examined for the presence of fluorescein. Without exception fluorescein was present in the petiole down as far as the region which had been scalded. The terminal leaflets were then removed, washed, and tested with iodine. In all of them the veins in the portion which had not been immersed in fluorescein showed a distinct starch test (fig. 3). The portion of the leaflet which had been immersed showed no starch whatsoever, possibly because of insufficient oxygen. The results indicate clearly, however, that carbohydrates did move into the leaflets while fluorescein was moving out.

Similar results were obtained from another simpler experiment. Fifteen terminal leaflets were darkened for three days as described above. Five of them were

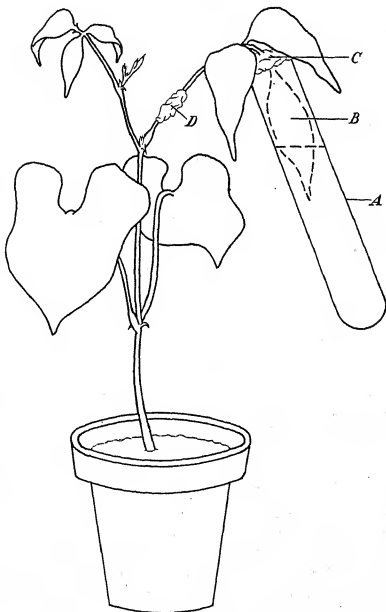


Fig. 1.—A. Dark chamber made by coating a test tube successively with black lacquer and aluminum paint.—B. Previously starved terminal leaflet with slightly scraped, distal portion immersed in fluorescein solution.—C. Cotton packing.—D. Cotton through which scalding water was percolated.

tested for starch and none was found. The lateral leaflets contained abundant starch. Each of these leaves appeared as that shown in figure 2. The remaining ten leaves were removed from the plant and taken to a dark room. About an inch was cut off the lower end of the petiole under water. The cut end was then immersed in a test tube of water. The midrib of the terminal leaflet was scraped lightly along the upper third of its length, and this portion of the leaflet was immersed in a test tube of fluorescein solution. After remaining two days in the dark room, the leaves were examined for fluorescein. In all of the leaves it had moved about half way down the petiole. The terminal leaflets were then tested

gradually loses every trace of its original supply (fig. 2).

DISCUSSION.—In the light of the behavior of these solutes in bean plants, it seems that those theories which propose a unidirectional mass flow of sieve tube contents to account for the mechanism of transport in intact plants can no longer be considered seriously. The demonstration that different solutes can move simultaneously in opposite directions in the same tissue corroborates considerable other evidence which has pointed to the same conclusion.

Among the prominent existing theories which propose mechanisms allowing this simultaneous contradiirectional transport, the one proposed originally by DeVries of protoplasmic streaming seems to fit the facts best. Mason, Maskell, and Phillis have rejected this theory as seeming "to be as untenable as the theory of a directed mass flow." This rejection resulted partly from a calculation of the theoretical rate of protoplasmic streaming necessary to transport materials at the observed rate in cotton plants. The calculation indicated that streaming at a speed of 336 centimeters per minute would be required to transport materials at their experimentally determined rate. This speed, as the writers point out, seems quite impossible. The calculation, however, involved the misapplication of a concentration gradient, which made their result twenty times too great. They found that

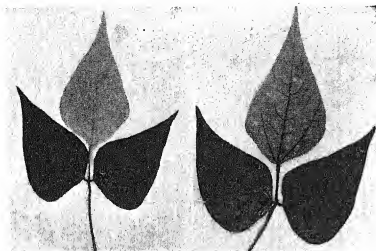


Fig. 2 (left). An iodine treated leaf, following a three day period in which the terminal leaflet had been darkened. N.B. Although the lateral leaflets are gorged with starch, there is no trace of it in the terminal leaflet, nor even in the rachis above the portion exposed to light.

Fig. 3 (right). An iodine treated leaf two days after the terminal leaflet, completely devoid of starch, had been placed in a dark chamber with its tip in fluorescein and the petiole scalded. N.B. Starch occurs along the entire rachis and along the larger veins.

for starch. In seven of the ten, starch was present in veins of the portion which had not been immersed (fig. 4). Probably the absence of starch in the other three resulted from a low original supply in the lateral leaflets, for in no case in other experiments, in which the lateral leaflets were exposed to light, did starch fail to appear in the terminal leaflets. These results corroborate those of the preceding experiments, and in addition it is noteworthy that the fluorescein moved into the lateral leaflets which were exporting sugar. Cross-sections of the rachis, examined microscopically, indicated that the movement of fluorescein took place in the phloem of these cut leaves just as it did in intact leaves.

To the writer it seems remarkable that with the entire cut leaf in complete darkness, the terminal leaflet receives sufficient sugar to form starch (fig. 4), whereas with the leaf in situ, with the lateral leaflets exposed to bright light and the terminal leaflet darkened, the latter not only fails to form starch but

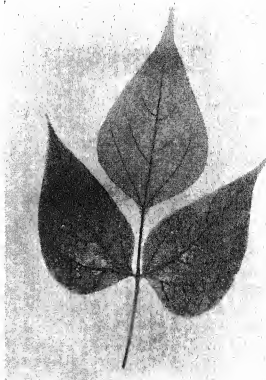


Fig. 4. An iodine treated leaf, originally treated exactly as that in figure 2, and then placed in a dark room for two days with the cut end of its petiole in water and the tip of the terminal leaflet in fluorescein solution. N.B. A darkened terminal leaflet loses all of its starch if the leaf is attached to the plant, even while the lateral leaflets are exposed to bright sunlight (fig. 2), but it receives sufficient sugar from the lateral leaflets in complete darkness to form starch if the leaf is cut from the plant, providing there is an abundant supply in the lateral leaflets when the leaf is cut.

with a concentration gradient of one gram per cubic centimeter per linear centimeter, the apparent diffusion constant in cotton was 0.07 grams per second per square centimeter of sieve tube groups. They pointed out that only half the pores in any sieve plate are concerned with transport in one direction and that since there are approximately twenty sieve tube elements per linear centimeter, the contents of each element differs in concentration from those next in series by 0.05 grams per cubic centimeter. Reasoning from these facts, the writers state that, "the net amount passing along under unit gradient will be $\frac{1}{2}R \times \pi \times 0.05$ grams per square centimeter per second, where R is the linear rate through the pores and π is the fraction of the sieve plate occupied by the pores." Since the writers express R in centimeters per second, they are obviously mistaken in using 0.05 in the calculation. The concentration gradient per centimeter remains at one gram per cubic centimeter; therefore, the net amount passing along must be expressed as $\frac{1}{2}R \times \pi \times 1$, and this should be equal to 0.07 grams which was determined for that gradient per linear centimeter. Calculated with this correction, the rate of streaming required is 16.8, not 336, centimeters per minute. If Mason, Maskell, and Phillis were correct in their calculation, the rate of streaming, with a constant concentration gradient, would vary directly with the number of sieve plates per centimeter, and if an infinite number of plates were assumed to be present, the rate of streaming would, of necessity, be infinitely rapid. Although this assumption is purposefully exaggerated, it emphasizes the fallacy in the statement quoted above, for it is apparent that the number of sieve plates per linear centimeter cannot affect the theoretical rate of streaming through the sieve pores. Even with this correction, the rate of streaming found is greater than any observed rate in plants. The calculation must not be considered too seriously, however, because the unavoidable errors in measurement and chemical analyses, when multiplied in a calculation of this nature, are more likely than not to distort the facts. A measurement of the cross-sectional area of the phloem in cut stems, for example, undoubtedly results in a value far smaller than the actual area in uncut stems, for, as Curtis (1935) has pointed out, cutting releases the normally high pressures in the phloem and results in a marked shrinkage or collapse of that tissue. The streaming hypothesis should not be discarded on the basis of such questionable evidence.

The theory of activated diffusion proposed by Mason, Maskell, and Phillis (1936), while it allows simultaneous movement of different solutes in opposite directions, sheds little light on the mechanism of transport. They assume, without structural evidence, the existence of an ultramicroscopic capillary system in sieve tubes which decreases resistance to diffusion to such an extent that solutes can travel through it at a rate more than twenty thousand times greater than their diffusion rate through water. Apart from the lack of structural evidence for this assumption,

it is much more probable that solute molecules are carried along with their solvent or protoplasmic matrix than that they speed through it independently. The mechanism involved in the diffusion-like movement of discrete particles at enormously high speeds is difficult to visualize, whereas the universally observed streaming movement of protoplasm with its resident solutes, although it is not thoroughly understood, is certainly far from the realm of conjecture. The postulation of protoplasmic streaming in mature sieve tubes, even though it has not as yet been observed, seems to the writer to be vastly safer than that of an entirely novel and hypothetical subvisible system that evades either proof or disproof. Both theories are consistent with most of the facts known from translocation studies, and, in addition, the streaming hypothesis proposes a mechanism, known to exist in the majority of plant cells, to account for the apparent "activation," or acceleration of diffusion.

Van den Honert (1932) succeeded in demonstrating a rapid movement of a solution of oleic acid, containing excess potassium hydroxide, along the interface between layers of ether and water. The rate of transport was 68,000 times greater than the diffusion rate of potassium in vitro. Van den Honert proposes tentatively that sugar in the plant may be converted into an "interface-active" form, and, lowering the surface tension between two liquid layers, spread rapidly along interfaces until "concentration differences are equalized." He suggests the boundary between the cell sap and protoplasm as a likely interface for this transport. As a result of the movement of materials along an interface, currents are generated in both the liquid layers; thus, according to this theory, protoplasmic streaming may be the result rather than the cause of rapid transport. The failure to observe streaming in cell sap in no way vitiates the theory, for it is entirely possible that the active interfaces occur within the protoplasm.

It was noted previously that fluorescein, when applied to a terminal leaflet, entered and passed rapidly down through the phloem of the leaf into the stem. It was also noted that in a cut leaf with its petiole immersed part way in water, the fluorescein traveled only about half way down the petiole. Skaer (1931) likewise found that cut leaves of several plants, including bean, emptied their carbohydrates extremely slowly. It does not seem likely that cutting a petiole with a razor destroys the interface between the cell sap and protoplasm several inches above the cut, and if the interface exists after the cut, fluorescein or sugars should, according to van den Honert's theory, continue to spread rapidly as long as differences in concentration exist. If the active interfaces are within the protoplasm, however, they might be altered for some distance by the pressure release resulting from opening the phloem. From these observations it seems that surface tension forces are not the only ones involved in the transport of solutes in plants, although they may quite possibly be involved with others in causing protoplasmic streaming.

Of these three theories of solute transport in plants, the one which seems to fit the observations made in this study most completely is that of protoplasmic streaming. The direction of the movement of each solute was independent of the other and apparently governed only by the direction of its concentration gradient. According to this theory one would also expect cutting a petiole to stop normal transfer for a considerable distance above the cut, since the release of pressure at the cut end presumably causes a cessation of streaming along the entire severed tube. Protoplasmic streaming alone, however, does not explain the marked polarity observed in the movement of many solutes. Both fluorescein and carbohydrates move much more rapidly downward than upward in bean plants. It can be seen in figure 2 that the sugar concentration in a darkened terminal leaflet remains below the threshold concentration for starch formation even though the two lateral leaflets of the same leaf are gorged with starch. The dry weight of these lateral leaflets decreases from eight to nine per cent

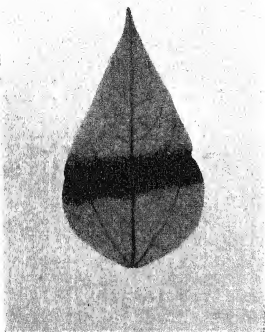


Fig. 5. An iodine treated terminal leaflet which was originally emptied of starch. A transverse band was then exposed to sunlight for two consecutive days. N.B. There is fully as much starch along the veins below the exposed area as above it.

during a 15-hour period in darkness (table 1, 2, 3), therefore it is apparent that carbohydrate export must take place rapidly. Since no starch appears in the terminal leaflet, most of the sugar exported by the lateral leaflets must move downward. This might be explained by the streaming hypothesis if one postulates that the storage cells in the lower portion of the plant absorb sugar actively and thereby preserve a steep concentration gradient from the supplying cells.

If this postulation were correct, the concentration of carbohydrates in the phloem below a supplying

region should be lower than that above the same region. To determine this, portions in the center of previously starved leaflets were exposed to normal sunlight for two days. This was accomplished by cutting rectangular areas from the sides of the envelopes in which the leaflets had been darkened. After the two-day period, the leaflets were tested for starch. A photograph of one of these is shown in figure 5. It can be seen that there is as much starch along the veins below the exposed area as above it. There is no reason to believe that the threshold concentration for starch formation differs in the two portions of the same vein; therefore it is apparent that the concentration of sugar in the phloem below is at least as great as that above the supplying region. In addition to streaming and active solute absorption, the distribution mechanism must involve other forces which result in polar movement. Without further investigation it would indeed be futile to postulate the nature of these forces. It is clear from the preceding data, however, that, whatever the forces are, they affect fluorescein and sugar independently, for the upward movement of fluorescein is not impeded by the downward movement of sugar, nor does the upward movement of sugar retard the downward movement of fluorescein.

The use of fluorescein in translocation studies may be criticized on the grounds that it is not a normal plant solute. Used in a dilution of one part to a thousand parts of water, it has no apparent harmful effects. Seedlings grown with their roots in a solution of that concentration appeared perfectly healthy, although they were slightly smaller than others grown in tap water. The use of fluorescein also may be questioned because it occasionally travels in the xylem more rapidly than in the phloem. When applied to a cut stem it moves upward in the xylem, or when applied to leaves of water-deficient plants it moves downward in the xylem. An attempt was made to demonstrate opposite movement of carbohydrates and fluorescein in the phloem of sprouts from potato tubers. Fluorescein applied at the tips of the shoots moved rapidly downward into the tuber through the xylem. Apparently whenever the water column of treated plants moves rapidly, fluorescein, entering the xylem presumably by diffusion, travels with it. In well-watered plants, however, the path of downward movement is restricted to the phloem. This can be demonstrated so easily that a laboratory exercise, for students in the physiology course here, has been devised which involves the determination of the path of its movement. Students invariably find it restricted to the phloem when their plants are well watered. In this same exercise students also demonstrate that carbohydrates can move in the opposite direction at the same time.

SUMMARY

The purpose of this work was to determine whether or not two different solutes can move simultaneously in opposite directions in the same phloem tissue. Fluorescein applied to the terminal leaflets of com-

pound bean leaves entered and moved through the phloem into the lateral leaflets. A comparison of the dry weights of the two lateral leaflets, one before and one after the treatment, indicated clearly that carbohydrates had moved out of the leaf while fluorescein was entering. During the course of the experiment, cross sections of petioles from similarly treated leaves were taken at random intervals of time; these invariably showed fluorescein present in the phloem. It is apparent therefore that the sugar and fluorescein did not move in opposite directions at alternate periods of time during the experiment. Fluorescein was likewise applied to starved terminal leaflets in darkened test tubes. The lateral leaflets of these leaves were exposed to sunlight and the petiole was sealed. Fluorescein moved out of the terminal leaflets, and after a two-day period sufficient sugar had entered to produce starch. Cut leaves with starch-filled lateral leaflets and starved terminal leaflets were

arranged in a dark room with the cut ends of the petioles in water and the tips of the terminal leaflets in fluorescein solution. Fluorescein moved out of the terminal leaflets through the phloem into the lateral leaflets and part way down the petioles, while sufficient sugar moved out of the lateral leaflets and into the terminal leaflets to produce starch there.

These results led to the conclusion that carbohydrates and fluorescein can move simultaneously in opposite directions in the same phloem tissue. If this conclusion is justified, it vitates those theories which propose a unidirectional mass flow of sieve tube contents. Of the remaining theories of solute transport, the one which seems to be most satisfactory is that of protoplasmic streaming.

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GEMMIPARY IN *KALANCHOE ROTUNDIFOLIA* AND OTHER CRASSULACEAE¹

Harry N. Stoudt

THE phenomenon of vegetative propagation in Crassulaceae has received much attention from students of morphology and physiology. A comparison of the morphological development of plantlets of *Kalanchoe rotundifolia* Harv. with other members of the family should aid in understanding more adequately the phenomenon so characteristic of the group.

Yarbrough (1936) discussed vegetative propagation in *Sedum Stahlii*. The leaves are attached to the stem by a short petiole. When the leaf is detached, a pseudocicatrice forms over the newly exposed petiolar surface or attachment area. A "cicatrice meristem" is formed several cell layers behind this region. The chloroplasts disappear from the apparently mature parenchyma cells of the petiole, and with the resumption of mitotic activity these cells add to the formation of the callus. Thus a callus pad of considerable size is formed, from which root primordia arise endogenously above five days after the leaf is detached. Shoot primordia arise exogenously in about ten days.

In a report on vegetative propagation in *Byrnesia Weinbergii*, the writer (1934) demonstrated that the sessile leaf of this plant produces a plantlet at its base. When the leaf is detached, a callus forms in the region where the leaf was attached to the stem. The plantlet, however, is formed from a residual meristem and not from the callus. No organ rudiments are differentiated in the basal region of the normal mature leaf until ten to twelve days after the removal of the leaf. When the dormancy of the meristem is broken, shoot primordia arise exogenously and root primordia arise endogenously.

Freeland (1933) discussed vegetative propagation in *Bryophyllum crenatum* in which plantlets develop from residual meristems in the notches of the parent leaf. He found that the amount of differentiation of plantlet rudiments varies. A stem primordium only may be formed by the time the parent leaf is mature and the foliar bud may remain dormant in this condition. When growth is resumed, the roots originate adventitiously from the stem of the plantlet beyond the parent leaf.

According to published accounts on *Bryophyllum calycinum* by Reed (1923), Howe (1931), Yarbrough (1932a, 1932b, 1934), Naylor (1932) and Freeland (1933), plantlets develop from residual meristems in the notches of the leaves. These regions are already differentiated into minute leaf, stem, and root primordia while the leaves are still attached to the plant.

During the summer of 1934 the writer made a histological study of vegetative propagation in *Kalanchoe tubiflora* and *Kalanchoe Daigremontiana*. In

the fall of that year Clamp (1934) described this process accurately in the former species, while Johnson (1934) described it for the latter species. Their results were similar to those already obtained by the author. Therefore, a detailed account of plantlet propagation in those two species will be omitted here. The cylindrical leaves of *Kalanchoe tubiflora* (Harvey) Hamet have an entire margin except at their apices where there are five to seven teeth. Abaxially between these teeth, claws develop upon which plantlets are formed. *Kalanchoe Daigremontiana* Hamet and Perrier also produces plantlets, or "pseudobulbils" as they were called by Johnson (1934), on leaf-claws which arise between the teeth of the serrate margin of the leaf. These plantlets are made up of at least two sets of leaf primordia and a shortened disc-like stem from which roots arise. In both species a residual meristem persists between the teeth. Leaf-claws and plantlets develop from this region, and the latter are often differentiated into leaf, stem, and root rudiments long before the parent leaf has attained its maximum size.

MATERIALS AND METHODS.—The original plants of *K. rotundifolia* Harv. (fig. 1) were obtained from the Botanical Gardens of the University of Michigan. External and histological examination of the development of plantlets was made in the summer of 1934 at the Long Island Biological Laboratory.

To study the histological development of the plantlets formed on the leaves, it was found necessary to investigate the anatomy of the leaves in the various stages of development—viz., from those in which the cells were entirely meristematic to those upon which mature plantlets had developed. These were fixed in formalin-acetic-alcohol and dehydrated by the butyl alcohol method as outlined by Zirkle (1930). Transverse, horizontal, and sagittal—i.e., longitudinal vertical—sections were cut ten microns in thickness. These were mounted serially, stained with Delafield's haematoxylin and counterstained with safranin as outlined by Chamberlain (1928). Drawings were made with the aid of a microprojection apparatus.

External morphology.—The leaves are opposite, fleshy, ovate, with the margin entire, and taper at the basal end to form a petiole (fig. 1). In a shallow groove on the adaxial side a protuberance is occasionally evident close to the point of leaf attachment. This protuberance is a plantlet primordium which consists of the primordia of two leaves and a stem. Development of these rudiments on the mature leaf then ceases until the leaf is separated from the parent plant, whereupon primordia of the plantlet resume growth (fig. 2). When root primordia are formed, the plantlet becomes independent, and the parent leaf disintegrates.

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Internal morphology.—To observe the earlier stages in the formation of plantlets from the meristematic regions, serial sections through the apical meristems of the plants were studied. Examination of a young leaf, composed entirely of meristematic cells, showed that the region where a plantlet is later to be formed is already evident.

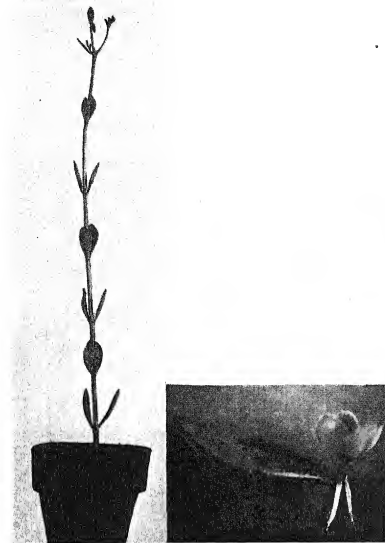


Fig. 1, 2.—Fig. 1 (left). Young plant of *Kalanchoe rotundifolia*.—Fig. 2 (right). Leaf of *K. rotundifolia* developing a new plant at the base of the petiole.

Transverse sections through a leaf, nine millimeters in length, and still attached to the stem, show that a mesophyll tissue is already differentiated and that the meristematic region at the base of the petiole has developed into a comparatively large mass of cells (fig. 3). This mass of undifferentiated cells forms a cushion on the adaxial surface of the petiole. Figure 4, a section through the petiole of the leaf opposite to that illustrated in figure 3, shows a slight differentiation into leaf primordia, as is evidenced by the protuberances on the edges of the meristematic cushion.

Transverse sections through the base of a petiole of a mature leaf (fig. 5) show a deeper groove on the petiole; the meristematic cushion is differentiated into two leaf primordia with a stem primordium between them. As yet, no root primordia have formed and the histological study of the petioles of leaves

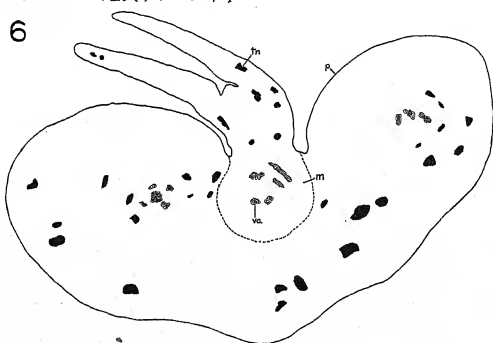
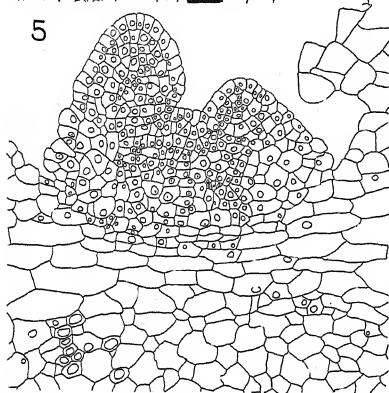
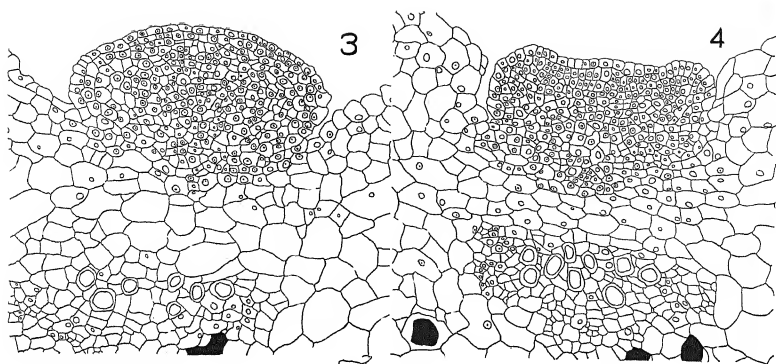
that had been placed on moist sand for eighteen days showed no signs of root formation. Figure 6, which is an outline of a transverse section through the petiole, *p*, shows the relation of the bud to the petiole. The dotted line represents the limits of the meristematic region, *m*. The central portion of this region surrounding the conducting cells, *va*, is composed of small cells and is encircled by larger meristematic cells. Tannin cells, *tn*, have formed throughout the bud and petiole. Figure 6 differs from figure 5 only in the greater development of the leaf and stem primordia.

To obtain root primordia, leaves were removed from the plants and placed on moist paper toweling in a covered culture dish. Sagittal sections (fig. 7) show a large meristematic cushion, *ch*. Several endogenous root primordia, *r*, develop on the region of the cushion nearest the base of the petiole, while numerous leaf primordia form at the opposite end.

It is significant that in this species leaf and stem primordia have developed on the normal mature leaf. The primordium, bud, then remains dormant, and root primordia are initiated after the leaf has been removed from the plant. Leaves placed in a moist chamber formed root primordia in ten days, but leaves placed on moist sand exhibited no signs of root formation in eighteen days.

Discussion.—In the different species of *Crassulaceae* at the time the parent leaf matures, there are various degrees of differentiation of organ rudiments in the meristematic cushion. These follow a definite sequence, ranging from species showing the undifferentiated state to species with complete plantlets. Thus the apparently mature parenchyma cells in the petiole of *Sedum Stahlii* resume mitotic activity and form a plantlet when the parent leaf is removed from the plant; in *Byrnesia Weinbergii* no organ rudiments are yet differentiated in the dormant meristem in the basal portion of the sessile leaf. The meristematic regions, located in the notches of the mature leaf of *Bryophyllum crenatum*, may be differentiated into a stem primordium only at the time the parent leaf is mature. Sometimes, however, leaf and stem rudiments may have formed. The petiole of the mature leaf of *Kalanchoe rotundifolia* reveals a meristematic cushion that is differentiated into two leaf primordia and a stem primordium. In *Bryophyllum calycinum*, root, stem, and leaf primordia form but are not always externally visible. Finally, in *Kalanchoe Daigremontiana* and *K. tubiflora* plantlets consisting of root, stem, and leaf primordia are visible macroscopically even before the parent leaf has attained maximum size. Summarily then, the leaves of all the foregoing species of *Crassulaceae* exhibit the same method of vegetative reproduction. Their differences are expressed in the stage of development attained by the meristems, or organ rudiments derived from them, at the time the leaves of the parent plants are mature.

Yarborough (1936) states that "the development of shoots and roots from leaves of *Sedum Stahlii*, which at the time of detachment possess no residual



meristem, is clearly an example of regeneration through the formation of adventitious structures." This seems not to be the usual conception of regeneration—i.e., the replacement of lost or injured parts. In *Podostemon ceratophyllum*, Hammond (1936) describes this process as follows: "if the distal end of a root is cut off, a new root growing point is initiated back of the cut surface" and "a shoot is regenerated at the distal cut end of a stem."

Yarborough (1936), in discussing vegetative reproduction in *Sedum stahlii*, is of the opinion that "this condition is to be sharply contrasted with that in *Bryophyllum*, etc., where true foliar embryos occur." That *Sedum stahlii* would seem to represent merely a primitive condition is further emphasized by the characterization of those cells that produced the plantlets as not being markedly specialized as to cell shape and wall thickness. These cells would seem to be only slightly different from cells of a meristematic nature such as is present in leaf bases of *Byrnesia Weinbergii*.

Degree of differentiation of the meristem in the mature leaf was used to form the series *Sedum stahlii*—*Byrnesia Weinbergii*—*Bryophyllum crenatum*—*Kalanchoe rotundifolia*—*Bryophyllum calycinum*—*Kalanchoe Daigremontiana* and *K. tubiflora* discussed above in which *Sedum stahlii* is primitive and *Kalanchoe Daigremontiana* and *K. tubiflora* most advanced. This series parallels the idea of evolutionary development of the various genera as held by Schönland (Engler & Prantl) (1894), who states that *Sedum* represents the oldest type of the *Crassulaceae*. In the same reference the floral leaves of *Sedum* are described as free or almost free, while the floral leaves are fused into a tube in *Kalanchoe*. The same holds true when reference is made to Hutchinson's (1926) criteria concerning free and fused parts of the inflorescence.

Since all leaves are derived from an apical meristem and, in their early stages, are composed of undifferentiated meristem, it is obvious that in certain species of the *Crassulaceae* certain areas, either at base or margin, may remain meristematic and may

retain the same potentialities as an apical (stem tip) meristem. The apical region of a stem is a direct derivative of the plumule and in this sense is always embryonic. In all the species investigated the root primordia arise endogenously either from stem rudiment or from a meristematic cushion. Stoudt (1934) pointed out in *Byrnesia* the similarity in origin and structure of plantlets developed from a residual meristem at the base of the leaf to the axillary buds. This similarity extended even to the endogenous formation of root primordia. Such roots are unquestionably adventitious in the latter instance and consequently can be so interpreted in the former. The term "gemmpary" was proposed for this particular method of vegetative reproduction of plantlets from residual meristems or buds. Since then, a reference worthy of note has come to the attention of the writer. Linnaeus (1824) uses the term in the same sense when he says, "Gemmatio est Gemmae constructio ex foliis, stipulis, petiolis, aut squamis." In the same reference he makes the statement, "Species Gemmarum variae sunt. Deciduae in *Dentaria*, *Ornithogalo*, *Lilio*, *Saxifraga*." Thus the use of the term "gemmpary" in this paper does not involve any extension of its original meaning or application.

SUMMARY

Detached leaves of *Kalanchoe rotundifolia* produce plantlets from residual meristems on the adaxial surface of the basal portion of the petiole.

By the time the leaf is fully mature, the meristematic cushion has become differentiated into primordia of two leaves and a stem. This bud then remains dormant, and root primordia are initiated after the leaf is removed from the plant.

The various species of *Crassulaceae* so far studied arrange themselves in a definite sequence in respect to the degree to which the meristematic cushion becomes differentiated by the time the parent leaf is mature.

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Fig. 3-7.—Fig. 3. Transverse section through base of petiole of a 9 mm. leaf, showing undifferentiated meristematic cushion.—Fig. 4. Similar section of opposite leaf, showing slight differentiation into leaf primordia.—Fig. 5. Transverse section through meristematic region of normal mature leaf, showing stem and leaf primordia.—Fig. 6. Outline of transverse section through petiole, *p*, showing its relation to the bud. The meristematic cells, *m*, surround the vascular area, *va*. Tannin cells, *tc*, are seen throughout the section.—Fig. 7. Outline of sagittal section of the petiole, *p*, showing the meristematic cushion, *ch*, from which arise root primordia, *r*. This is not a median section for leaf and stem primordia.

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ROOT RESISTANCE AS A CAUSE OF THE ABSORPTION LAG¹

Paul J. Kramer

It is well known that during times of rapid transpiration a decrease in moisture content of plants usually occurs, frequently accompanied by loss of turgor and wilting of the leaves and other succulent parts. Separate, but simultaneous, measurements of the rates of transpiration and absorption indicate that during periods of moderate to high transpiration more water is being lost from the plant than is being absorbed (Livingston and Hawkins, 1915; Kramer, 1937). Since this occurs even in plants with their root systems in moist soil or tap water, it must result from an inadequate root surface for absorption, resistance to the absorption of water in the plant itself, or inability to translocate water from roots to leaves with sufficient rapidity, rather than because of a deficient supply to the roots.

The present investigation was begun in an attempt to determine the cause of the lag of absorption behind transpiration. When the rate of transpiration is rapidly increased, as in the early morning hours, evaporation from the mesophyll cells bordering the intercellular spaces increases their suction tension. This causes movement of water into them from the adjacent cells, which are in turn supplied with water from the xylem of the leaf veins. Rapid removal of water from the xylem in the veins sets up a tension which extends down through the water-conducting system of the stems into the roots and directly or indirectly causes an inflow of water across the root cortex from the soil into the conducting system. Wilson and Livingston (1937) have discussed the adjustments which occur in the water-conducting systems during changes in rate of transpiration. A lag might be caused by resistance to water movement at three stages in its passage from soil to leaves. The first stage is in its movement from the soil or surrounding solution across the living cells of the root into the xylem. The second is in its movement up through the xylem of root, stem, and leaves, and the third stage is in its movement from the xylem of the leaves

through the mesophyll cells to the evaporating surfaces. Wilson and Livingston (1937) found that in plants of basket willow less than ten minutes were usually required for the propagation of hydrostatic pressure readjustments from the transpiring membranes of the leaves to the solution surrounding the roots. They calculated that of the total lag only 5 per cent for 30 cm. stems and 15 per cent for 120 cm. stems was to be attributed to stem resistance, the remainder being required to overcome resistance in the roots and leaves. They suggested that most of the lag probably occurs in the cells of the leaves.

Unpublished data obtained in this laboratory indicate that the diurnal moisture changes occurring in leaves are quantitatively much larger than those occurring in roots, which ordinarily show small variations in moisture content. It is well known, however, that if the stems of wilted plants are bent over and cut off under water the leaves will usually recover in a few minutes. The recovery frequently is so rapid that the wilted leaves can be seen opening out and rising to their normal condition, and the entire process requires less than 5 minutes. Wilted plants with intact roots require considerably more time to recover following thorough watering. This fact seems to indicate that the tissues of the roots offer considerable resistance to the absorption of water. Several series of experiments therefore were performed to measure the root resistance.

EXPERIMENTAL METHODS.—Sunflower and tomato seedlings were grown in a nutrient solution until they were 30 to 40 cm. in height and had well developed root systems and several pairs of leaves. Plants were then placed in potometers improvised from 500 ml. wide-mouth Erlenmeyer flasks fitted with rubber stoppers. Each stopper contained three holes, one near the center for the stem of the plant and two others near the margin on opposite sides. In one of these marginal holes was placed a small separatory funnel which was used in refilling the potometer between measurements. In the other marginal hole was placed a capillary tube graduated in 0.01 ml. divisions so that it could be read to 0.005 ml. and having a total capacity of 3.5 ml. Each stopper was split from the margin to the central hole on one side in order

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to place the stem in the central hole without injury. The plants were sealed into the holes in the stoppers by grafting wax, the potometers were filled with tap water and were then placed in a water bath maintained at the desired temperature. The volume of water absorbed per unit of time was readily determined by observing the position of the meniscus of the retreating water column at the desired intervals.

RESULTS.—After the potometers had come to the temperature of the bath and the plants had attained a uniform rate of absorption, the tops were covered with a cardboard cylinder for 10 minutes to decrease transpiration, the rate of absorption being recorded for each minute. The cover was removed and the rate of absorption observed and recorded for another 10-minute period. Covering the plants probably slowed down transpiration immediately; uncovering them certainly resulted in an immediate resumption of transpiration. After a few minutes the process of covering and uncovering was repeated. The roots were then cut off under water with a sharp razor and the potometer reset, this operation requiring about one minute. The rate of absorption of the top was recorded for the next 20 minutes after removal of the roots, by which time it had returned to a constant rate. The top was then covered and uncovered two or three times just as had been done before removing the roots and the rate of absorption recorded for each minute during the entire cycle of operations. The average of 15 determinations on seven sunflower plants is shown in figure 1.

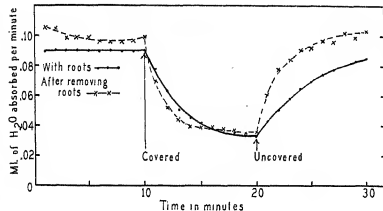


Fig. 1. Effect of removing roots on absorption lag accompanying changes in rate of transpiration. The rate of absorption was found to be very significantly increased by removal of the roots as tested by Student's method. The response of absorption rate to change in transpiration rate was also significantly more rapid in plants without roots than in plants with roots.

It is quite evident that the lag was much smaller after the roots were removed. When transpiration was slowed down by covering the tops, the rate of absorption by the plants with roots attained equilibrium in about 10 minutes, but after the roots were removed, equilibrium was practically attained in 5 minutes. The 10-minute observation period following uncovering of the plants was too short for the absorption of rooted plants to return to normal, but after their roots were removed, the original rate was

attained in 8 or 10 minutes. It should also be noted that the average rate of absorption was significantly higher after the roots had been removed than it was while they were still attached. These facts indicate that removal of the roots actually facilitated the absorption of water by removing the resistance to movement offered by the living cells of the cortex. When the resistance to movement offered by the root cortex is removed, the lag period is greatly reduced because no time is required for readjustment of the hydrostatic pressure in the cells of the cortex. The rate of absorption therefore responds much more rapidly to changes in rate of transpiration.

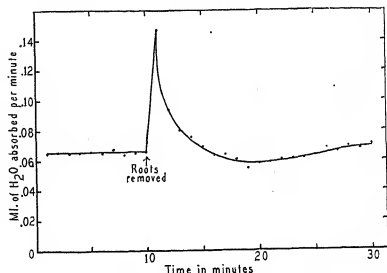


Fig. 2. Effect of removing roots on rate of absorption of sunflower plants at 25°C.

The resistance to movement offered by the root cortex at 25°C. is indicated in another manner by figure 2, which shows the greatly increased rate of absorption immediately following the removal of the roots. Evidently the mesophyll cells of the leaves were in a state of incipient wilting and the water in the xylem was under tension, presumably because it was not passing across the root cortex into the conducting system as rapidly as it tended to be removed from the leaves in transpiration. Removal of the roots eliminated the resistance offered by the cortex, resulting in a very rapid inflow of water during the first minute or two after cutting off the roots. The rate of absorption rapidly fell as the cells of the leaves regained their turgidity, until it attained a new equilibrium as high as or higher than the original rate (compare rates before and after removal of roots in figure 1).

Both of the above experiments were performed on tomato plants with similar results.

It was found that the resistance to the passage of water through the tissues of the root was greatly increased at low temperatures. Potometers containing sunflower plants were placed in a water bath at 6°C., and within 30 minutes the plants were wilted and their stems and leaves definitely drooping. After approximately one hour in the bath the rate of absorption of these plants was recorded for 10 minutes; the roots were then removed and the rate recorded for another 20 minutes. The average result of 15 such determina-

tions on sunflower plants is shown in figure 3. Immediately after removing the roots, absorption increased nearly 10 times, but the rate soon fell to equilibrium at about 2.5 times the rate before the roots were removed. As shown in figure 3, this high rate of absorption was maintained for at least an hour after removal of the roots. All evidences of wilting disappeared within two to five minutes after removal of the roots.

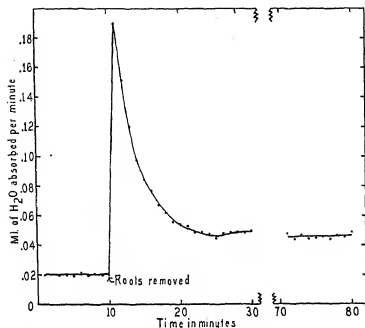


Fig. 3. Effect of removing roots on rate of absorption of sunflower plants at 6°C.

The wilting of these plants when their roots were cooled evidently was caused by increased resistance to the passage of water through the cooled cortex. As suggested by various writers and most recently by Arndt (1937), increased viscosity of the water itself and increased viscosity of the protoplasm are doubtless both concerned in the slower intake of water at low temperatures. This increased resistance to water movement is probably the most important cause of the inability of plants to absorb adequate quantities of water at low temperatures.

The magnitude of the resistance to water movement offered by the cortical tissues of the roots as compared to the resistance in the conducting system of the stem was shown even more strikingly in another manner. The tops of a group of six large sunflower plants were removed about 35 cm. above the roots, and graduated pipettes were attached by rubber tubing to the stumps while the root systems were immersed in flasks of water maintained at 25°C. in a water bath. The pipettes were attached to a vacuum pump, and a pressure gradient of 60 cm. of Hg was maintained from outside to inside of the system while the rate of water intake was recorded for several 30-minute periods. After the rate had attained equilibrium, the roots were removed and the rate of intake through the stems was measured for intervals of 6 minutes. From these data the average rate of intake for a 30-minute period was calculated. The results are shown in table 1.

TABLE 1. Average rate of water intake for 30 minute period before and after removal of roots.

Roots attached	Roots removed	Percentage increase
0.183 ml.	15.2 ml.	8,206.0%

Over 83 times as much water was absorbed after the roots had been removed as while they were attached.

This experiment also indicates that the resistance to water movement in the stem of sunflowers is very low. The writer has measured water movement under pressure through segments of woody and herbaceous stems of several species. While it is comparatively high in some woody stems, it is quite low in all the herbaceous stems which were investigated. In this and various similar experiments a pressure gradient of less than one atmosphere caused water movement to occur at a rate far in excess of any that could occur in a transpiring plant. Resistance to flow through the stem certainly is not an important factor in the absorption lag of herbaceous plants and probably not in some woody species, as indicated by the data of Wilson and Livingston (1937) for willow.

Discussion.—The results of these experiments indicate that the tissues of the root offer considerable resistance to the passage of water. This is not surprising in view of the fact that water must pass through the epidermis, several rows of cortical parenchyma cells, the endodermis, and the pericycle. The endodermis is frequently composed largely of cells with radial walls made impermeable by the suberized Casparian strips. In such cells all water must pass through the protoplasts, but this restriction is probably considerably lessened by the presence of unsuberized passage cells opposite the xylem points. Some of the water doubtless moves along the radial walls from cell to cell in the cortex, but most of it probably moves through the protoplasts, crossing only the tangential walls. While the cell walls offer some resistance, most of it is probably caused by the layer of more or less viscous protoplasm lining each cell. It has been shown (Renner, 1929; Kramer, 1933) that with the same pressure much more water passes through dead root systems than through the same root systems while alive. Killing the roots would certainly affect the structure and permeability of the cytoplasm and its membranes to a much greater extent than the cell walls. Since most of the pathway from epidermis to xylem is across the cortex, it is probable that most of the resistance to water movement is produced by the protoplasm of the cortical cells. This is in accord with the views of Huber and Höfler (1930), who studied the permeability of cells to water by the rate of plasmolysis. They concluded that most of the resistance to water movement is offered by the protoplasm rather than the cell walls and that the resistance to water movement in root and leaf parenchyma is therefore largely produced by the protoplasts of the cells.

The combined effects of the resistance offered by all these cell membranes is to cause absorption to lag

behind transpiration. Transpiration may increase rapidly during the early hours of daylight, but absorption does not increase rapidly until sufficient tension is produced in the water-conducting system to overcome the resistance of the root cells to rapid passage of water. During this interval before absorption becomes equal to transpiration, the water not supplied through the roots is supplied from the cells of the plant itself.

In considering the results of these experiments it should be kept in mind that absorption rates after removal of roots always represent minimum rather than maximum values. It seems impossible to remove the roots without some plugging of the xylem, and even when care is used, stems occasionally become plugged at once, and the plants wilt. When the roots are cut off, some of the contents of cut cells are swept into the vessels, and bits of broken roots and other debris may also be carried in by the rapid flow of water immediately after cutting. Air bubbles frequently accumulate and plug the vessels, and enzymatic and bacterial action may result in gum formation and plugging after a few hours. If these sources of error could be avoided, greater increases in rate of absorption following removal of the roots would be obtained. Furthermore, these experiments were performed in a laboratory under artificial light where the rate of transpiration is not as high as it would have been out of doors. A few experiments performed out of doors with very high transpiration rates indicated that much greater temporary increases in absorption occur following removal of roots from rapidly transpiring plants in full sunlight, probably because of a greater initial tension in the hydrostatic system at the time of cutting under such conditions.

Since the rate of absorption was frequently as high or higher after removal of the roots as while they were attached, it seems that they could not have been actively secreting or "pumping" any appreciable quantity of water into the xylem vessels. If the forces bringing about most of the absorption were being developed by the cells of the roots, then removal of the roots should have resulted in decreased water intake. Evidently most of the water is absorbed through rather than by the roots—that is, the forces bringing about absorption originate in the shoots, and the roots for the most part act simply as absorbing surfaces. The intake of water in transpiring plants probably occurs because of a gradient of decreasing pressure from the water in the soil to that in the xylem vessels. This gradient results from the state of tension existing in the hydrostatic system of transpiring plants. Such an explanation of course requires a vigorously growing root system which is continually bringing the absorbing surfaces into contact with hitherto uninvaded portions of the soil.

SUMMARY

Experiments were performed to determine why absorption lags behind transpiration in plants which have adequate root systems and an abundant supply of water. It was found that the lag of absorption

behind transpiration is greatly decreased in sunflower and tomato after the roots have been removed. It was also found that the rate of absorption of transpiring plants was greatly increased temporarily by removing the roots. The rate of absorption rapidly returned to a rate approximating or exceeding the rate prior to removal of the roots. The rate of water intake through sunflower stems attached to a vacuum pump was increased 83 times by removing the roots, indicating that the root resistance is much greater than the stem resistance.

When the roots were removed from plants having their roots at 6°C., the rate of absorption temporarily increased nearly tenfold and then fell to a rate 2.5 times the rate prior to removal of the roots. The tops which had previously been badly wilted regained their turgor within five minutes following removal of the roots.

These results indicate that the living cells between epidermis and xylem offer considerable resistance to the passage of water and are probably responsible for a large part of the lag of absorption behind transpiration at normal temperatures. This resistance is much greater at low temperatures than at high temperatures, probably because the viscosity of both protoplasm and water increases as the temperature decreases. The increased resistance to water movement is believed to be the principal reason for the inability of plants to absorb sufficient water at low temperatures.

The conducting system of sunflower stems offers very little resistance to water movement. Stem resistance is probably a negligible factor in causing absorption lag in herbaceous plants and likewise in some woody species.

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Aven Nelson

***Pachylophus prolatus* Aven Nelson, sp. nov.**

Probabiliter perennis pluribus caulibus assurgentibus e radice magno friabili; pubescentia copiosa molli plus minusve lanosa decidua superne sed in partibus inferioribus caulium petiolis marginibus foliorum capsulisque permanenti; caulibus crassis foliosissimis 1-3 dm. longis; foliis valde longis (ad 2.5 dm.), laminis anguste oblongo-lanceolatis in petiolum angustatis, marginibus undulato-dentatis, laminis petiolisque subaequalibus; floribus in axillis, albis quam foliis brevioribus; capsulis stipitatis circa 5 cm. longis parce tuberculatis.

Probably a short-lived perennial, the root large and friable, with several assurgent stems from the crown; pubescence abundant, softly lanate and somewhat floccose and more or less deciduous, permanent especially below on the stem and on the petioles, the leaf-margins and the capsules; stems thick, 1-3 dm. long, assurgent, very leafy throughout, the lower petioles persisting; leaves very long, up to 2.5 dm., the blades narrowly oblong-lanceolate, irregularly undulate-dentate, tapering into the petiole, which is often margined in part and with an occasional tooth, blade and petiole subequal in length; flowers axillary, shorter than the leaves, the hypanthium tube slender, up to 15 cm. long, somewhat dilated above; the flower characters not diagnostic being those common to members of the genus; the calyx-lobes lance-linear, about 25 mm.; corolla white, fading pink; capsule stipitate, about 5 cm. long, tapering toward the apex, scarcely tuberculate.

The genus *Pachylophus* originally contained only acaulescent plants. A few, however, have since been found that are definitely caulescent. This fact does not invalidate the genus, for among the several segregates of *Oenothera* none other is more readily and unmistakably recognized in the field by its aspect, while its flower and fruit characters are singularly distinctive.

The species now proposed recalls *P. eximius* Gray, but it surpasses that not only in the length of its stem but in its extraordinarily long leaves and corolla tube. *P. eximia* is erect; *P. prolatus* prostrate. In *P. eximia*, as noted by Dr. Gray, the capsules are strictly sessile in the leaf-axils, while in *P. prolatus* they are evidently both pedicellate and stipitate.

Secured on a dry sandy foothill near Globe, Arizona, by the writer, no. 10300, May 4, 1925. (Type: Ry. Mt. Herb.)

Additional specimens secured in the same locality in 1935 bear out the diagnosis as given.

***Phlox acerba* Aven Nelson, sp. nov.**

Caulice lignoso ramoso; ramis gracilibus fasciculatis ad intervalia orituris 10-20 cm. altis caulibus prostratis; foliis linearibus 8-15 mm. longis subulatis acerosisque; flore solitario terminali, calyce 10 mm. longo lobis ciliatis; corolla laete rosea, tubo longo, lobis 4-6 mm. longis obovato-crenulatis; capsula oblongo-ovata loculis 1-ovulatis.

¹ Received for publication October 25, 1937.

Crown more or less branched, the woody root spreading horizontally and simulating a rhizome from which at intervals new clusters of one or more stems may spring; stems 10-20 cm. high, simple or with short branches from the nodes, the main axis and the branchlets one-flowered; leaves pale-green, 8-15 mm. long, narrowly linear, erect or spreading, involute-thickened, moderately rigid-subulate, terminating in a firm sharply aciculate prickle as do also the calyx lobes; pubescence of stem short-hispid, scarcely glandular, on leaves and calyx-lobes sparse, short and crisp; calyx about 10 mm. long, teeth ciliate-margined below, the tube and teeth subequal; corolla pink, its tube well surpassing the calyx teeth, its lobes obovate, crenulate, 4-6 mm. long; styles short, about half as long as the corolla tube; capsules oblong-oval, the cells 1-seeded.

Not readily comparable to any other species. By using a given single character one might "key" it out to *P. austromontana* Cov., or even to *P. pinifolia* or *P. variabilis* Brand. The two latter are, however, of a different alliance. In habit and in the slender erect stems it stands apart. The flowers of *P. acerba* are nearly sessile, the short very uniform leaves continuing to those that involucre and conceal the very short petiole under the calyx.

Secured by Aven Nelson and Ruth A. Nelson in a side draw of Oak Creek Canyon, north-central Arizona. It was not abundant, occurring in small scattering clumps in the open woods (pine and oak), its rhizome-like roots spreading through the loose soil of the forest floor. The collectors at once became aware of the aculeate character of the short needle-like leaves. No. 2119 is the type (Ry. Mt. Herb.).

***Gilia ashtonae* Aven Nelson, sp. nov.**

Planta ramis pluribus bifurcis patentibus 15-20 cm. alta plerumque latior; caulibus ramisque gracilibus; pedunculis pedicellisque filiformibus; foliis hispidulis oppositis linearibus vel trifidis acerosis; floribus inflorescentiae paniculae singulis vel in cymis; corolla lutea infundibuliformi, tubo brevis, lobis obovatis; antheris inclusis; stylo exserto; ovario ovato. Habitu *G. filipes* Benth. Sect. *Linanthus*.

Growth habit of *G. filipes* Benth. and therefore of the section *Linanthus*: stems dichotomously profusely and widely branched, 15-20 cm. high and often wider than high, stems, branches, and branchlets slender, with peduncles and pedicels filiform; herbage sparsely hispidulose as is also the calyx; leaves opposite, short (1 cm., more or less), linear-acerose, mostly 3-parted; inflorescence paniculate, the flowers borne singly or in few-flowered cymes; pedicels filiform, often 3 cm. or more long; calyx-tube narrowly conical, its teeth lance-subulate, acerose, about as long as the tube; corolla golden-yellow, 9-12 mm. long, funneliform, its very short tube and the longer throat equalling the calyx, its limb divided to the calyx-tips, the obovate lobes not widely spreading, as long as

the throat and tube together; anthers at the orifice of the throat, the slender style and the long linear stigma equalling the corolla lobes; ovary oval.

This handsome species was secured by the junior member of the field party (consisting of Mrs. Nelson and myself). I name it in honor of Ruth Ashton Nelson, botanist, who shares equally in the labors and the joys of botanical exploration, and whose alertness, industry, and discrimination more than double the efficiency of our expeditions.

This plant was secured but once and so may be rare. "Tortilla flat," sandy banks of a little canyon stream, about 3 miles east of Canyon Lake, on the "Apache Trail," Arizona, May 3, 1935, no. 1768. (Type: Ry. Mt. Herb.)

Gilia inconspicua lacerata Aven Nelson, var. nov.

Robusta alterne ramosa; foliis amplis paucis tenuibus viridibus dipinnato-laceratis, lobis latis saepe partitis calycibus capsulisque distentis.

Plant stout, erect, 4-6 dm. high, sparingly glandular-ciliate-pubescent, main axis with a few stout, long, alternate branches; leaves large, few, thin, green, mostly basal, and at the base of the 2-4 larger branches, the larger ones 8-12 cm. long and 3-5 cm. broad, all variously bipinnately lacerate into broad irregular lobes, sometimes cleft to the midrib but often only part way. The lobes acerose-cuspidate; flowers somewhat smaller than in the species but otherwise characteristic, the capsules and distended calyces conspicuous.

No. 1424, of the collections made by Aven Nelson and Ruth A. Nelson in the Tanque Verde Mts., near Tucson, Arizona, Apr. 7, 1935.

In some respects it suggests *G. incisa* Benth. and *G. latifolia* Wats., or even *G. scopulorum* Jones, but it lacks the villous glandulosity of that smaller plant with its larger yellow-throated corolla.

Plagiobothrys micranthus Aven Nelson, sp. nov.

Parva annua hiemis ramosa semi-prostrata; foliis fere radicalibus anguste oblongis 2-3 cm. longis vel brevioribus; calyci albo-hispido 2-3 mm. longo; corolla inconspicua 3-4 mm. longa; nuculis 4 ovato-quadrangulatis, ciliatris transverso-oblongo.

A small semi-prostrate winter annual, mature fruit in April in the type locality; branching from the base, very leafy at the base, less so on the short slender stems and leaves wanting or nearly so on the floriferous portion; green in aspect but short hispid hairs abundant; leaves mostly narrowly oblong, obtusish, 3-2 cm. long and shorter; flowers small, the calyx white-hispid; calyx lobes lance-triangular, short, not connivent, the whole calyx in fruit not more than 2-3 mm. long; corolla inconspicuous, the tube and limb subequal, scarcely surpassing the calyx or the lobes spreading out over the calyx-tips; nutlets 4, ovate-quadrangulate with short beak, 1-1.5 mm. long and broad, minutely papillose, the lateral and dorsal ridges similar, transverse lines irregular and inconspicuous, attachment-scar transversely oblong, well elevated above the crest of the ventral keel.

Seemingly most closely allied to *P. canescens* Benth. (or some variety of that) but smaller in every way with harsher pubescence and with nutlets characteristically different.

Secured by the writer on moist stream banks near Prescott, Ariz., April 28, 1925, no. 10232. (Type: Ry. Mt. Herb.)

Stachys limitanea Aven Nelson, sp. nov.

Perennis in fissuris humidis scopulorum; caulibus robustis assurgentibus 1 m. longis vel longioribus viscoso-resiniferis; pubescentia longa mollique; internodiis inferioribus 1-2 dm. longis superioribus brevioribus; foliis pubescentibus trigono-ovatis 5-8 cm. longis supra saturate viridibus subtus pallidioribus, marginibus denticulato-serratis; floribus verticillatis; calycibus campanulatis; corollis coccineis 14-20 mm. longis bilabiatis, labio superiore integro inferiore profunde diviso.

Perennial, in moist crevices of steep stony slopes; stems assurgent to erect, up to 1 m. or more in length, moderately stout, pubescent with long soft spreading ciliae below, less so upward, very obscurely resiniferous-viscid below the indument throughout, including all the parts of the inflorescence in which the cilia have been mostly replaced by a dense puberulence: internodes long, up to 2 dm. below, gradually shortening upward through the open spicate inflorescence: leaves narrowly triangular-ovate, denticulate-serrate, dark green above, paler beneath, short-pubescent above, densely so beneath, subacute, rounded, truncate or subcordate at base, the lower and larger up to 8 cm. long, gradually smaller upward and passing into the sessile foliar bracts of the verticils; the lower petioles long, equalling or exceeding the blades, shorter upward: verticils mostly 4-flowered; calyx deeply and narrowly campanulate, its tube 5-6 mm. long with 5 triangular acute cuspidate teeth half as long; corolla scarlet, its tube twice as long as the calyx, strongly bilabiate, the upper lip entire, broadly ovate or subreniform, 7-8 mm. in diameter; lower lip deeply divided, the middle lobe ovate, the lateral oblong-spatulate.

There are very few species in this genus with scarlet corollas, perhaps only two north of the Mexican border—*S. chamissonis* Benth. and *S. coccinea* Jacq. The proposed species seems intermediate between these but differs markedly from both in the character and distribution of its pubescence. It lacks the retrorse scabrous pubescence of the angles of the stem of the former and the fine puberulence of the latter. Through Mexico southward, the genus has larger representation but among the scarlet-reds only the two already named appear to be known from northern Mexico.

The type and collection number is 1471, by Aven Nelson and Ruth A. Nelson, Apr. 12, 1935, at "Massacre Camp," in the mountains between Ruby and the Tucson-Nogales highway, only a few miles from the Mexican border.

Penstemon amplus Aven Nelson, sp. nov.

Caulibus foliosis fasciculari robustis assurgentibus 6-10 dm. altis puberulis; foliis amplis pubescentibus

ovato-lanceolatis 8-12 cm. longis vel longioribus; petiolis longis; foliis superioribus sessilibus non perfoliatis; inflorescentiis spicato-racemosis, cymis paucifloris congestis puberulis viscoso-glandulosisque; corollis coccineis 18-25 mm. longis tubuloso-infundibuliformibus bilabiatis, lobis brevibus erectis; staminibus exsertis; filamentis sterilibus inclusis subglabris.

Stems several, clustered on the large crown, stout, assurgent, 6-10 dm. high, grayish-puberulent throughout, very leafy from the base up to the inflorescence where the leaves are abruptly reduced to foliar bracts that gradually diminish to lance-subulate remnants: leaves large, pubescence similar to that of the stem but usually less dense; the leaves of young sterile shoots, 12-20 cm. long, the blade ovate to ovate-lanceolate or narrower, 8-12 cm. long on petioles of the same length; 2-3 pairs of the stem-leaves also petioled, similar in shape, the next succeeding pairs sessile, with rounded clasping base but not perfoliate, 10-15 cm. long, tapering regularly from the wider base to the acute tip; inflorescence spicately racemose, the few-flowered cymose clusters crowded on the long axis ($\frac{1}{4}$ - $\frac{1}{2}$ of the height of the plant). The flowers usually crowded over to one side, and thus seemingly unilateral; calyx and pedicels rather harshly and densely puberulent, the hairs obscurely subviscid-glandular; calyx lobes ovate, abruptly acute, only 3-4 mm. long, narrowly margined at the widest part; corolla deep scarlet, 18-25 mm. long, narrowly tubular-funnelform, the tube merging gradually into the throat; the limb bilabiate but short and erect, its lobes broadly oblong, the longer (upper lip) about 5 mm. long; stamens well exserted as is also the slender style; sterile filament shorter and included, its filamentous tip with a few (3-5) well defined hairs; the anthers horseshoe-shaped, obversely saccate—i.e., open to the free tips and saccate at the connective end. (The open end in the dry state suggests merely a pore for the discharge of the pollen grains.)

The proposed species has so much in common with *P. exsertus* A. Nels., Amer. Jour. Bot. 18: 438, as to raise the question of its status. Its pronounced puberulence, especially in the inflorescence and the almost glandular-viscidness on the calyx (glabrous in *P. exsertus*), the larger size in every way, and the difference in the sterile filament suggest specific rank.

***Penstemon mirus* Aven Nelson, sp. nov.**

Caulibus fascicularibus robustis puberulis semi-erectis 5-8 dm. altis, parte superiore tertia florifera; foliis crassis 5-7 jugis lanceolatis inferioribus petiolatis superioribus sessilibus; floribus amplis late purpureo-roseis 3 cm. longis; calycis lobis ovatis 5 mm. longis; corollis glabris tubis brevibus, faucibus longis ventricosis 12-15 mm. diametro, bilabiatis lobis latis; antheris omnino dehiscentibus non explanatis; filamentis sterilibus parvis pubescentibus.

Densely but minutely grayish-puberulent, especially below, less so in the inflorescence but there obscurely glandular, the calyces mostly glabrous: stems several from the crown, stout, simple, assurgent-erect, 5-8 dm. high (possibly more), the floral portion about

one third of the whole: leaves thick, in 5-7 pairs, the lower 2 or 3 pairs oblong, on petioles usually shorter than the oblong-oval blade; the mid-stem leaves largest, triangular-lanceolate, broadest at the rounded or sub-cordate sessile base, 8-14 cm. long, the narrowing tip scarcely acute; inflorescence narrow and appearing 1-sided but the few flowered (2-3) axillary cymes in pairs, peduncles and pedicels equally variable, 1-3 cm. in length; flowers large and showy, brilliant purplish-rose, about 30 mm. long; calyx short, its lobes about 5 mm. long, ovate, abruptly acute or short-acuminate, narrowly scarious-margined; corolla glabrous without and within, the tube scarcely longer than the calyx, rather abruptly expanded into the long ventricose throat which at its widest part may be up to 15 mm. in diameter (usually a little less), limb bilabiate, the lips nearly equal or often the lower seemingly much shorter due to the occasional reflexing of the broad rounded lobes; anther-cells opening throughout but not through the junction, neither divergent nor explanate; sterile filament sparsely pubescent on the upper half with soft unequal hairs, most abundant on the flattened tip.

This may be a hybrid between *P. Palmeri* Gray and *P. exsertus* A. Nels. Both of these were present in the immediate area where *P. mirus* was secured. The corolla of *P. mirus* is much like that of *P. palmeri*, though less expanded; the sterile filament is less hairy, and it is wholly glabrous on the lower lip within. It has the herbage and stem puberulence of *P. exsertus*. The leaves, in shape, are a compromise between those of the two hypothetical parents. Could the blending of cream-white and pink in *P. palmeri* with the scarlet-red of *P. exsertus* produce in the corolla the brilliant purplish-rose of *P. mirus*? In any event, this striking and seemingly well established plant needs a name. Viewed from the ear window, it suggested a huge clump of "glorified" *P. glaber* Pursh, a species that probably does not occur in Arizona.

The type, no. 2076, was secured in Oak Canyon, in N. central Arizona, on May 22, 1935, by Aven Nelson and Ruth A. Nelson.

***Penstemon shantzii incognitus* Aven Nelson, var. nov.**

Caulibus gracilibus assurgentibus 7-10 dm. altis; inflorescentiis paniculatis 3-5 dm. longis; floribus ut in *P. Shantzii* sed minoribus.

Undoubtedly closely related to the species but of a very different aspect: stems slender, assurgent-erect, 7-10 dm. high, floriferous for half their length; leaves longer and slenderer and mostly quite erect; the inflorescence cymose-paniculate, with many flowers on elongated peduncles and pedicels, these quite variable in length and giving a decidedly paniculate appearance to the long clusters of well distributed flowers: floral characters as in the species but all the parts somewhat reduced in size (cf. *P. Shantzii* A. Nels., Amer. Jour. Bot. 23: 270. 1936).

Secured in the Papago Indian Reservation, near Sells, Arizona, by Aven Nelson and Ruth A. Nelson, no. 1373, Mar. 31, 1935.

Gutierrezia polyantha Aven Nelson, sp. nov.

Caudice ramosissimo caulis gracilibus simplicibus basi ramosis superne scabro-viscidis; ramulis gracilibus; capitulis solitariis vel glomeratis amplis subglobosis 3 mm. altis; squamis involucri oblongis scariosis nitidis apice erosis; flosculis radii 10, flosculis disci circa 20; paleis linearilanceolatis; stylis exsertis.

Perennial, the short shrubby branches of the caudex giving rise to the numerous slender stems which are simple below but paniculately branched above; the herbage only obscurely granular-scarbous-viscid; the ultimate branchlets slender, leafy, monocephalous or, more often, with two or more subglomerate large heads: leaves linear, divaricate and variously flexed or curved, 2-4 cm. long: heads large, subspherical, 5 mm. (more or less) in diameter; bracts oblong-oval, scarious, shining, with the greenish tip, obscurely viscid around the minutely erose obtuse tip; flowers many (for this genus); rays about 10; the disk about 20; paleae linear-lanceolate, shorter than the corolla tube; the anthers and the longer styles well extruded.

The general aspect of this plant is much the same as *G. sarothrae* but less viscid and with a very different involucre both in shape and color. The large and relatively few heads carry the largest number of flowers ever reported in this genus. The average seems to be as above, but some of the larger heads have up to 12 rays and disk flowers up to 24.

Secured only once on the recent extended field trip made by Aven Nelson and Ruth A. Nelson. Field no. 1638, secured a few miles north of Tucson, Arizona, April 26, 1935. (Type: Ry. Mt. Herb.)

Hymenoclea hemidioica Aven Nelson, sp. nov.

Frutex humilis ramosus 1 m. altus vel altior; ramis ramulosis floriferis numerosis gracilibus in summa parte; inflorescentis bifurcatis in plantis diversis, alteris compactis omnino masculinis capitalis in corymbis numerosis, alteris strictis minus compactis capitulis masculinis feminisque admixtis; foliis linearibus 1-2 cm. longis; capitulis masculinis numerosissimis viridibus pallidis, flosculis 3-5; involucre fertili 4 mm. longo apice in rostrum producto, squamis 6-7 radiatopatentibus cingente, omnino patentibus 6-8 mm. diametro.

A low freely branched shrub, 1 m. or more high, the main axes breaking up at the top into numerous slender floriferous branchlets forming a broad compact corymbose inflorescence in the staminate plant and a narrower more open one in the monoecious individuals; herbage pale-green, glabrous or obscurely puberulent; leaves linear, slightly involute, only 1-2 cm. long, or many of them bractlike; staminate heads very numerous, only 3-5-flowered, pale-green or lighter; pistillate heads in small terminal clusters, accompanied by a few small staminate heads singly or in clusters on the branchlets below the terminal clusters of fertile flowers; fertile involucre about 4 mm. long, conical above the single whorl of median scales which are 5-7 in number, these are slightly broader than high, the fully expanded whorl 6-8 mm. in diameter.

One of the interesting things about this species is that it seems to indicate that this genus is on its way

to dioeciousness. Numerous individuals were wholly staminate; the monoecious individuals were either growing intermingled with or nearby in the same area and habitat (sandy, gravelly banks of a dry creek bank). In these, the pistillate heads were the conspicuous part of the trusses, but there were also many staminate heads. Possibly some day some one will find plants that bear only pistillate flowers.

But the hemi-dioeciousness is not the only character on which the species rests, as the characters given attest. Its short leaves and few-flowered heads at once attract attention.

Ordinarily the plants in this genus are *monoecious*—staminate and pistillate heads intermingled in the inflorescence in various ways. On seeing the proposed species in the field, one's attention is immediately attracted to the smooth and beautiful uniformity of the staminate individuals. The monoecious individuals have the usual aspect, except that the staminate heads are relatively few in the inflorescence.

Specimens were made from both kinds of plants. No. 1340, from the monoecious individuals; no. 1341 from those bearing staminate flowers only. The locality is on the banks of dry washes on the plains east of the Mohawk Mts., a few miles east of Mohawk, Arizona, where on March 29, 1935, much excellent material was secured.

Piptothrix arizonica Aven Nelson, sp. nov.

Frutex ramosus 30-60 cm. altus puberulus; foliis oppositis integris vel minute denticatis glabratibus oblongo-lanceolatis 2-4 cm. longis, petioliis 1-2 cm. longis; inflorescentiis axillaribus corymbis compositis; capitulis numerosis 5-6 mm. altis; involucri cupulati squamis lanceolatis striatis apice acutis, exterioribus 8-10 interioribus paucioribus; flosculis 30 vel ultra ochroleucis; achaeniis 4-5 angulatis fuscis; setis pappi 10-16.

Low slender-branched shrub, less than 1 m. high; the older part of the stems brown, glabrous, leafless, the younger part brown-purplish and leaf-bearing, finely puberulent in the inflorescence and on the newer leaves; leaves opposite, nearly entire, the teeth small and few, glabrate, oblong-lanceolate, the petioles 1-2 cm. long, the blade 2-4 cm., 1 cm. or less broad; inflorescence consists of peduncled compound corymbs from the axils of the uppermost leaves and the foliar bracts; heads numerous, 5-6 mm. high and nearly as broad, on slender pedicels, the whole appearing as a crowded, more or less leafy panicle, at the end of the branches, 1 dm. or less long; involucre low cup-shaped, bracts lanceolate, striate at base, acute or sub-acuminate, the inner row of 8-10, the outer fewer and shorter; flowers numerous (30 or more), ochroleucous (tallying closely with the original generic characterization; achene 4-5-angled; dark or almost black; pappus bristles more than 10 (12-16).

The six previously known species of this interesting genus are all Mexican. It is not strange that one should be found in southern Arizona, the climate and physical characters of which duplicate closely some of the Mexican areas.

The proposed species seems most nearly related to *P. Goldmannii* Robins. (Proc. Amer. Acad. 35: 323.

1900). Type no. 1567, Aven and Ruth Nelson, Apr. 17, 1935, in the foothills, west side of the Baboquivari Mts., Arizona. (Type: Ry. Mt. Herb.)

Cirsium arcuatum Aven Nelson, sp. nov.

Biennis 1-2 m. alta lanosa; caule basi robusto superne patenti-ramoso; ramis longis gracilibus longius; foliis amplis fere radicalibus lanceolatis runcinnatis decurrentibus 3-4 dm. longis maturitate glabris, capitibus amplis 5 cm. altis latisque; squamis involucri tenuibus arachnoideis interioribus erectis exterioribus recurvatis; flosculis numerosis gracillimis purpureis; acheniis 5 mm. longis glabris; pappo plumoso; foliis squamisque spinis debilibus praefixis.

Biennial (?), 1-2 m. high; stem stout at base but diminishing in size as long branches are given off at increasingly shorter intervals upward; that is, the trunk deliquesces into a very open wide-spreading plant, the long slender stems drooping under the weight of the large solitary terminal heads; stems and branches moderately lanate but not spiny; leaves mostly basal, large, up to 3-4 dm. or more long, lanceolate or oblanceolate, tapering into margined petioles, blade flat and becoming glabrate and green, runcinately lobed and toothed, each principal vein terminating in a weak straight spine 4-7 mm. long;

stem leaves small, decurrent on the stem, the spines similar but appearing more crowded: heads large, up to 5 cm. or more in width and height; bracts of the involucre thin and flat, lightly involved in arachnoid wool, the tips of the outer series sharply refracted and bearing spines similar to those of the leaves; the inner series erect, acuminate and purple-tipped and not spinose; flowers very numerous and slender, purple; achenes plump, 5 mm. long, shiny-glabrous; pappus softly plumose almost to the tip of the bristles.

I find nothing with which this is comparable. Conspicuous as the plant is, it seems to have been overlooked. It is not plentiful but was observed in several places in the area west from Tucson to Ajo and north to Superior and in the Salt River canyon. There were usually only a few plants in a given place—mostly on ditch banks or road grades but occasionally on gravelly bottom lands near streams. The long slender arched swaying branches, each with a single large head, necessarily attracted attention.

Type no. 1740, Apache Trail, near Canyon Lake, May 3, 1935, Aven Nelson and Ruth A. Nelson. (Type: Ry Mt. Herb.)

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CYTOLOGY OF PHYMATOTRICHUM ROOT ROT OF COTTON SEEDLINGS GROWN IN PURE CULTURE¹

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THE root rot disease of cotton, caused by *Phymatotrichum omnivorum* (Shear) Duggar, has been the subject of numerous field and laboratory investigations relating to the morphology and physiology of the fungus, the determination of its host range, and methods of control. Studies of the cytology of host-parasite relations have been included in the investigations of Peltier and Samson (1926) and Butler (1935). Peltier and Samson have studied the histology of infection under field conditions and recognized for cotton and alfalfa four avenues of entry of the fungus into the roots—namely, through lenticels, through wounds, between the cells of the outer cork layers, and at the points of emergence of lateral rootlets where rupture of the cortical tissues of the parent root occurs. According to them, the plectenchymatous fungous strand grows over the outer cork cells, after which the central hyphae of the strand proliferates in the direction of the root, sending out hyphae which form a mycelial mass. This mass is then said to force itself in wedge-like fashion between the cells of the host until the cork cambium is reached. From this point hyphae grow between (later through)

the cells of the cortex and finally invade the vascular cylinder, where they may enter and grow longitudinally through the tracheids. The wedge-shaped mycelial mass is said to be inserted by mechanical force between the cells of the root. The figures given by Peltier and Samson in support of this theory of penetration are perhaps open to other interpretations which might better answer questions concerning the basis from which is exerted the considerable force necessary to insert the mycelial mass and the lack of apparent trauma in the contiguous root cells. Butler has studied the penetration of watermelon roots by the mycelium of *P. omnivorum*, both under field conditions and following the inoculation of uncontaminated seedlings with pure cultures of the fungus. He reports a wedging of hyphal masses between the cells of the host, but fails to demonstrate clearly that this actually takes place. In the seedling roots he finds that fungous invasion takes place "by direct penetration into a cell, by growing between two epidermal cells and finally into a cell, and by the wedging of several or many hyphae, thus causing a lesion." Butler emphasizes the role of mechanical force in the penetration process, but states also that "apparently a softening action took place at the point of entry in addition to the mechanical pressure." An account of the penetration of tree roots by the rhizomorphs of *Armillaria mellea* has been given by Thomas (1934), who states that in addition to the mechanical force

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exerted by the advancing rhizomorph, as evidenced by the crushing of adjacent host cells, there is apparently a chemical action upon the cell walls of the root. This view has recently been supported by Leach (1937) in his account of *Armillaria* root rot of tea and various forest trees in Nyassaland. Henderson (1937) reports that cotton seedlings grown in filtrates from liquid cultures of *P. omnivorum* are retarded in growth and postulates from this the liberation of soluble toxic substances by the fungus.

The physiology of host-parasite relations in various infections brought about by parasitic fungi has been studied repeatedly since the pioneer work of de Bary (1886) and Marshall Ward (1888) by Brown (1915, 1916, 1917, 1934, 1936) and his associates, by Harter and Weimer (1923), and by many others. Brown (1934, 1936) has recently given thorough reviews of the subject. Such fungi as *Botrytis cinerea*, which cause rapid or generalized rotting of plant tissues, have been shown to invade the host at least to a great extent through the agency of an enzyme, pectinase, secreted and exuded from the growing hyphal tips, and perhaps by toxic substances which would account for the killing of host cells in advance of fungal penetration noted by de Bary. Brown and others have shown that the epidermal cuticle of leaves and stems is not acted upon by hyphal exudates, but is penetrated mechanically by slender processes from the appressoria.

In the work reported here, the problem of host-parasite relations in the *Phymatotrichum* root rot of cotton has been investigated by means of cytological technique in an attempt to determine the course of hyphal invasion of the cotton root and to indicate from direct or indirect evidence something of the mechanism employed by the fungus in its attack. The case has been intentionally simplified as much as possible by the use of uncontaminated cotton seedlings growing in vitro on the surface of nutrient agar and inoculated with fragments of pure culture sclerotia. The infections thus obtained are admittedly not strictly comparable to the disease on plants in the field, but certain advantages result from the use of young roots of simple structure inoculated with known material and observed in vitro during all phases of infection. The information gained from this study should have an important bearing upon the interpretation of data derived from the histological studies which are being carried on at present with naturally infected cotton roots.

METHODS.—The method of obtaining material for this study was similar to that used by Butler in his work on pure culture infections of watermelon seedlings. Cotton seeds of the variety Startex, which had been delinted with concentrated sulphuric acid, were surface-sterilized by immersion for one minute in 1:2000 mercuric chloride in 25 per cent ethyl alcohol, washed thoroughly in sterile distilled water, and placed on sterile potato-dextrose agar in Petri dishes. After about 48 hours germinating seeds with hypocotyls from one to two centimeters in length were

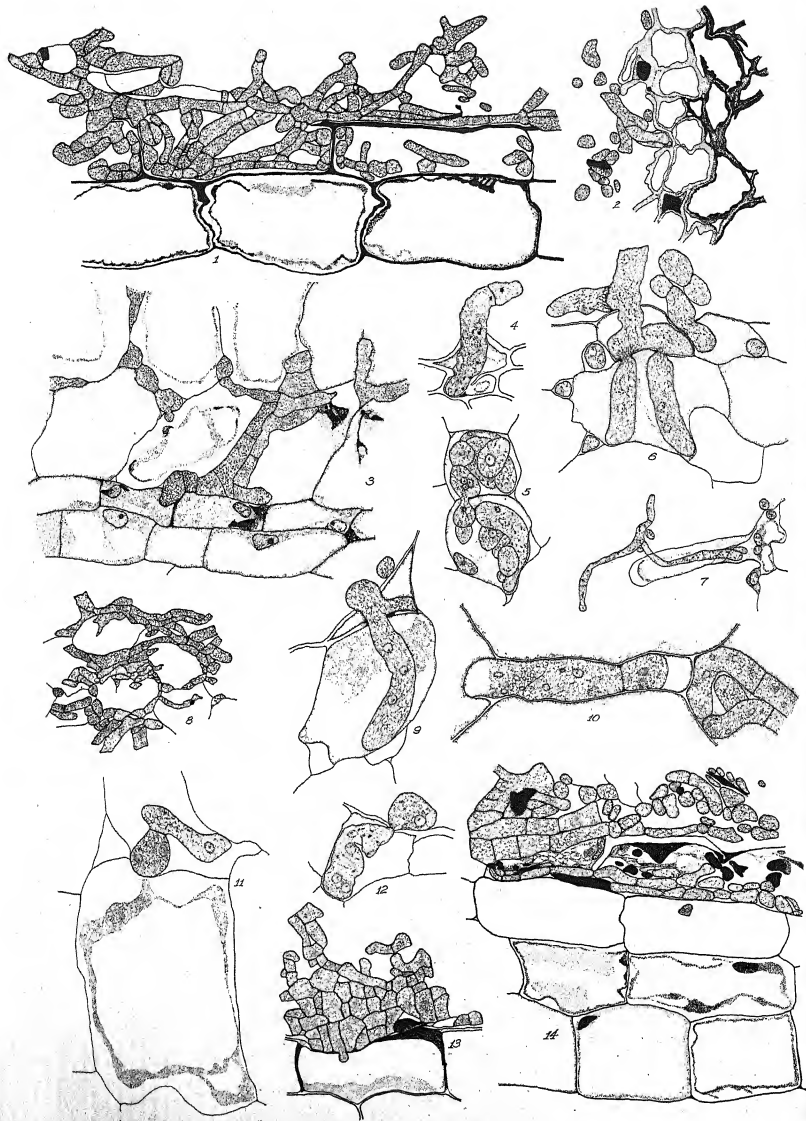
selected from plates in which no contamination had appeared and removed with flamed forceps to sterile potato-dextrose agar slants, one seedling to each slant with the hypocotyl directed toward the bottom of the tube. Upon each such slant at a distance of one to five millimeters from the hypocotyl was then placed a small fragment of a sclerotial mass from a pure culture of *P. omnivorum*. From 50 to 100 such tubes were prepared in every lot used in this work. The seedlings in a few tubes were left uninoculated to provide criteria of the typical structure of the cotton seedling root grown under these conditions.

After the preparation and inoculation of a given lot of seedlings, the tubes were incubated at room temperature and observed under low magnification several times daily for two weeks. At 24-hour intervals during that time the seedlings were removed from a few tubes and the roots cut off and fixed in Allen's modified Bouin's solution. All fixed material was embedded in paraffin, sectioned at 8-12 microns, and stained in Heidenhain's iron alum-haematoxylin with or without a counterstain of orange G.

EXTERNAL APPEARANCE OF ROOTS DURING INFECTION.—During the first 24 hours after inoculation the sclerotial fragments send out numerous radiating white hyphae, some of which extend toward the seedling root and make contact with it. The rapidly growing roots may curve in various directions, so that the original distance between inoculum and root is variously altered. Some rootlets bend immediately downward and continue through the agar, while others grow directly over the surface, or arch upward. Thus in any lot of tubes the times of first contact between hyphae and rootlets varies considerably. Some contacts are established during the first 24 hours, others not until many hours later after the root hairs have been formed.

Frequently the freshly cut sclerotial fragment liberates a yellowish-brown substance which is absorbed by the agar beneath, resulting within a few hours in a more or less circular discoloration of a radius of perhaps one-half centimeter about the inoculum. Roots which come into contact either with the cut surface of the sclerotium or with the discolored agar are usually inhibited in further growth and occasionally develop sunken brown necrotic areas even before being attacked by hyphae. In other cases the roots continue to appear healthy until more or less surrounded by the enveloping hyphal agglomeration.

In the first stages of attack a few individual hyphae grow over the surface of the root, branching and mingling with the root hairs where these have developed. During the first 24 hours after contact the hyphae surround the root and extend longitudinally, forming a dense superficial weft, which is at first white but later becomes light brown or buff-colored. The meristematic tip may continue growth for several days after the weft is established above. After five to ten days the plant is usually killed by the infection, which comes to involve all parts of the primary



and lateral roots. Beneath the weft the tissues are killed, showing dark-colored, sunken lesions.

MICROSCOPIC CHARACTERISTICS OF ROOTS DURING INFECTION.—Sections of roots fixed 24 to 48 hours after the beginning of attack show a gradual increase in the number of hyphae accumulated around the outside of the epidermis. The loose hyphal weft in section shows scattered fragments of hyphae, some of which run for short distances in contact with the epidermis (fig. 1, 14). At this stage there is no evidence of hyphal penetration. The weft usually becomes increasingly dense until a closely packed, pseudoparenchymatous mycelium is formed about the epidermis (fig. 13, 14). In such cases the cells of the epidermis and the outer two or three layers of the cortex begin to appear distorted, with thickened cell walls, shrunken protoplasts, and abnormal dark staining granular accumulations within the cells (fig. 1, 2, 13, 14). Later in the process the outermost walls of a few epidermal cells break down (fig. 1, 14) and hyphae from the surrounding fungal layer enter and occupy the empty cell cavities. In later stages the entire epidermis is destroyed and similarly the successive layers of the cortex until the endodermis is the outermost layer still intact. The process appears to be one of killing the protoplasts and breaking down the cell walls by some substance absorbed by the root in advance of hyphal penetration. The earliest evidence of cellular disintegration often appears as much as four cell layers removed from the nearest hyphae. None of the preparations of this type of material showed any evidence of the employment of mechanical force by the fungus.

In the type of attack just described, which characterized about half of the material examined, the entry of a hyphal tip into an intact root cell was rarely observed. Another process of invasion involves the direct penetration of individual hyphae into the epidermal cells, including root hairs, and into all tissues beneath. In this case also the hyphae form a loose weft around the root, as described above, and enter into close association with the root hairs when these are present. A number of instances have been seen in transverse sections through the root hair zone in which a single hypha had penetrated the wall of the hair and was growing through the lumen toward the base of the cell (fig. 7, 19). This process is possibly a characteristic mode of entry of the fungus into the

epidermis of this region. At the same time hyphal tips may penetrate the walls of other epidermal cells (fig. 4, 6, 9, 12, 18). As far as could be determined the hyphal tip forms no structure comparable to an appressorium, nor does it as a rule thrust an attenuated peg through the wall. One case was encountered (fig. 12) in which a hypha after having entered an epidermal cell still showed a marked constriction at the point of entry. Usually, however, hyphal entrance is effected with only a slight decrease in the diameter of the penetrating tube.

From the cells of the epidermis hyphal tips penetrate the walls of adjacent cortical cells and proceed into the interior of the root (fig. 6, 9, 18). In the progressive invasion of the cortex the growing, ramifying hyphae make their ways through the spaces between cells (fig. 3, 10, 17) and with apparently equal ease pierce walls and invade cellular cavities (fig. 5, 17). Hyphae which grow through the intercellular spaces frequently bring about a localized separation of contiguous layers of the wall (fig. 3, 10), perhaps through the dissolution of the pectic substances of the middle lamella. In many cases one or more hyphae appear to grow along the plane of the cell wall and destroy or replace it; at least a careful examination of such cells failed to show any evidence that the wall had merely been pushed to one side. As the macerating action continues, the intercellular mycelium becomes increasingly complex until each cell comes to be separated from those adjacent to it by a synnematos network (fig. 8). Hyphae that enter the cortical cells may either branch variously within the cell cavity or proceed from cell to cell, piercing the walls with slight or in many cases no constriction.

In the later stages of invasion of the cortex the cells first penetrated often become filled with branching, coiled hyphae of increasing complexity (fig. 5). When a group of cells is completely filled with mycelium, the remaining cell walls begin to break down, and the resulting mass of packed hyphae may obliterate all traces of original host tissue continuity.

From the cortex hyphae usually enter the endodermis (fig. 3), from which after more or less branching they invade the pericycle and the vascular tissues (fig. 17). Hyphae have frequently been observed in the lumina of tracheids in roots fixed 7 to 12 days after inoculation (fig. 15, 20). The mode of penetration of the tracheid walls seems to vary. In several

Fig. 1-14.—Fig. 1. Hyphal weft destroying epidermal layer and entering broken cells. The cortical cells beneath have been affected by the proximity of hyphae. $\times 862$.—Fig. 2. A group of hyphae apparently growing into a group of dead root cells. $\times 350$.—Fig. 3. Portion of a longitudinal section of a root showing hyphae in the cortex and one hyphal tip entering a cell of the endodermis. $\times 431$.—Fig. 4. Hyphal tip which has passed through epidermal cell and entered the cell beneath. $\times 862$.—Fig. 5. Two cortical cells in the fifth layer beneath the epidermis, showing both cells filled with hyphae and a single hypha penetrating the common wall. $\times 862$.—Fig. 6. Hyphae entering epidermal cells; the one at left has sent two branches into the underlying cell. $\times 862$.—Fig. 7. Mycelium which has entered a root hair. $\times 350$.—Fig. 8. A group of several cortical cells showing hyphae tending to follow the outlines of cell walls. $\times 175$.—Fig. 9. Hypha penetrating two outer cells of root. $\times 862$.—Fig. 10. A portion of wall between two adjacent cortical cells. A hypha has grown between the layers of the wall and appears to be pushing into the cell at the left. $\times 862$.—Fig. 11. A plasmolyzed cell of the cortex with hypha in adjoining cell. $\times 862$.—Fig. 12. Hypha penetrating an epidermal cell with constriction at the point of entry. $\times 862$.—Fig. 13. Pseudoparenchymatous hyphal mass in the process of destroying a cell of the cortex. $\times 350$.—Fig. 14. Hyphal mass occupying the outermost layer of the cortex. The epidermis has been broken down and almost completely absorbed. $\times 350$.

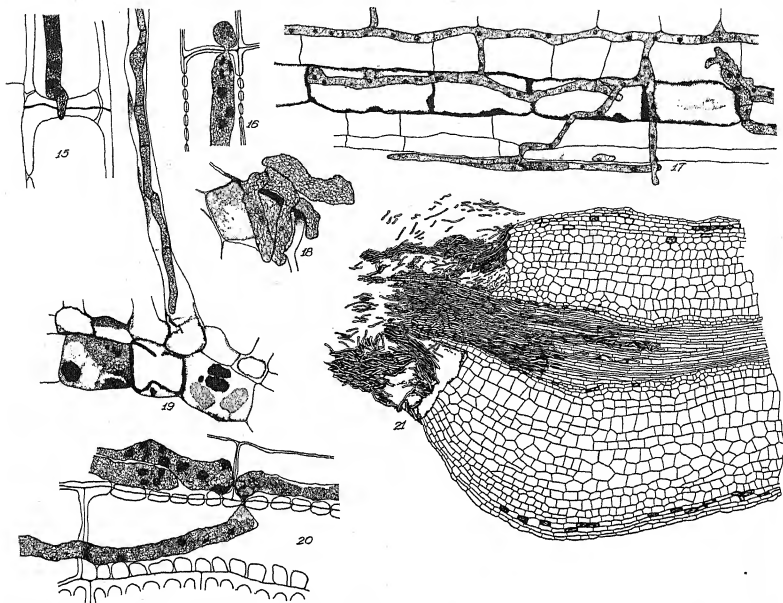


Fig. 15-21.—Fig. 15, 16. Penetration of hyphae through the end walls of tracheids. $\times 562$.—Fig. 17. Hyphae growing in cortex (above), entering and growing through endodermis layer (central darker layer), and passing into the pericycle (below endodermis). $\times 350$.—Fig. 18. Hyphae passing across an epidermal cell and entering an adjacent cell of the same layer. $\times 862$.—Fig. 19. Hypha in lumen of root hair. $\times 350$.—Fig. 20. Hyphae in tracheids. One hypha has grown through a pit in the wall. $\times 862$.—Fig. 21. Semi-diagrammatic drawing of longitudinal section of root. The tip has been rotted away by the fungus. Hyphae have grown further through the central cylinder than through the cortex or along the outside. Note dark-staining fragments of broken-down host tissue among hyphae. $\times 27$.

cases observed there was only a slight constriction of the hypha (fig. 15, 20), while in others the constriction was more marked (fig. 16, 20). A single hypha was observed apparently penetrating the membrane of a pit, with extreme attenuation at the point of passage (fig. 20). Peltier and Samson observed hyphae of *P. omnivorum* in the tracheids of field infected cotton roots and suggested that the vascular tissues may generally serve as a channel for the lengthwise growth of the fungus through the root. Some further suggestion of this possibility is seen in the present material in roots whose tips have been entirely destroyed by the fungus (fig. 21). In such cases the fungus is observed to have progressed farther through the central cylinder of the remaining unrotted part of the root than through the cortex or along the outside of the epidermis. The elongated cells of the vascular tissue perhaps promote more rapid growth because of the fewer cross walls. The

walls of the endodermal cells appear to resist the destructive action of the fungus longer than those of other root tissues, for in these examples of terminal decay the endodermis is the last layer to suffer disintegration.

The statement of Peltier and Samson that the fungus can enter the root at the point of origin of lateral roots is borne out also in seedling material. Many examples were seen of hyphae growing in the cortical rupture surrounding the bases of lateral roots. Such hyphae often extended in as far as the endodermis.

REACTION OF ROOT CELLS TO HYPHAL INVASION.—In the mass type of mycelial attack first described above, very few cases of actual cellular penetration by individual hyphae were observed. The fungus definitely exerts an action in advance and causes the death of cells several layers removed from the fungus. The appearance of the affected cells suggests strongly

the liberation of toxic substances from the mycelium. When first affected, the nuclei of such cells assume an abnormally homogeneous appearance and begin to lose the characteristic chromatic reticulum. Often the nucleus becomes irregular in shape (fig. 3, 14, 19). The primordial utricle shrinks irregularly from the cell wall, and the cytoplasm becomes filled with dark-staining granular aggregations (fig. 1, 14, 19). Near the fungous layer the cells are often empty, with the walls broken at various points. After the mycelium occupies an empty layer of epidermal or cortical cells, fragments of cell walls may occasionally be seen among the hyphae (fig. 14).

In the second type of invasion, characterized by the penetration of individual hyphae into the root tissues, there is to some extent the same action in advance of the hyphae. In the case of root hairs attacked by hyphae it is difficult to judge to what extent abnormalities are attributable to fungous action, since these fragile structures are usually greatly distorted in the preparation of permanent mounts. In other cells of the epidermis and in the cortex abnormalities in cell structure were observed which were clearly caused by the proximity of one or more hyphae. Many cells into which a hyphal tip had entered were devoid of cytoplasm or contained masses of dark-staining granular material. Moribund endodermal cells usually appeared yellow and suggested the presence of resinous contents. It is doubtful that any root cells invaded by or in proximity to hyphae contain living protoplasm.

The cotton seedling roots grown under the conditions stated above gave no evidence of possessing any protective or limiting mechanism to inhibit the fungus in its attack and spread. There was no indication of cork or other resistant layers produced by the host to protect its still undamaged tissues. The endodermis, probably the tissue most resistant to destruction, was readily penetrated by hyphae.

DISCUSSION.—The fungus *Phymatotrichum omnivorum* is capable of growth on a wide range of artificial media and dead organic substances. Its great adaptability to varying conditions in artificial culture finds a parallel in the huge number of plants which it is known to parasitize. Taubenhaus and Ezekiel (1936) have recently listed over 1700 species of dicotyledonous plants as more or less susceptible to *Phymatotrichum* root rot.

Fragments of sclerotial masses from pure cultures apparently liberate from cut surfaces a substance inhibitory to the growth of the cotton seedling root and capable of killing the root tissues. No attempt has been made in the present study to correlate this effect with the nutritional conditions under which the sclerotia were produced or to test for a similar effect from sclerotia produced in nature. It should be borne in mind, however, that in the preparation of material for this study the sclerotia were produced in pure culture on agar extremely rich in starch and sucrose, as well as mineral nutrients, and that during infection the hyphae were supported by potato-

dextrose agar. Further studies on the nature, quantities, and conditions of activity of enzymes and possible toxic substances produced by *P. omnivorum* under different environmental conditions should not only afford an explanation of the apparent widespread enzymic activity which is manifest in the infected roots studied here, but should be of great importance in accounting for the wide host range and variations in degree of susceptibility within it.

Hyphal invasion of cotton seedling roots under the conditions stated above is conceivably a process combining both mechanical and chemical activity on the part of the fungus. The formation of an encircling web may indicate that this means is taken in lieu of appressoria to establish a firm attachment prior to a hyphal thrust through the host cell walls. It seems more probable, however, that the hyphae merely accumulate around the outside of the root until their exudates have exerted a significant effect upon the immediately underlying host cells. The occasional constrictions in hyphae at points of passage through host cell walls could indicate mechanical force as a factor in penetration, though not necessarily so, since the constrictions rarely attain the high degree of attenuation usually associated with this mode of entry. The indirect evidence from cytological preparations is indicative of the strong part played by enzymic exudates. Thus may be explained those cases of erosion of successive layers of root tissue without penetration by individual hyphae. As far as could be observed, the hyphae never associate with living host plasma in anything approaching symbiosis, and moribund root cells were frequently seen as much as four cell layers away from the nearest hypha. The roots of young cotton seedlings are definitely absorbing organs and hence may unavoidably take in fungous exudates and be subject to a deleterious action in advance of hyphal entry.

It does not appear probable that the action of *P. omnivorum* is solely chemical, but chemical processes appear to play the dominant part in the rotting of host roots. No evidence has been seen here of the existence of mycelial wedges inserted by mechanical force into the root tissue.

SUMMARY

A cytological study of *Phymatotrichum omnivorum* infections in the roots of cotton seedlings grown and inoculated under pure culture conditions is reported. The fungous hyphae accumulate over the surface of the seedling root until a considerable web is formed. Sections of roots fixed during this and subsequent stages show that the external agglomeration either brings about the death and breaking down of the epidermis and successive layers of the cortex until only the central cylinder is left, or sends in individual hyphae which penetrate the epidermal cell walls with little or no constriction and ramify through and between all cells of the cortex, endodermis, and pericycle and are ultimately seen in the tracheids of the xylem. In the case of either process there is evi-

dence of chemical action by fungous secretions upon the host cells. No evidence was seen of the "mechanical wedge" type of penetration which has been reported for *Phymatotrichum* root rot of cotton under field conditions.

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INHIBITIONS DUE TO GROWTH HORMONES IN FERN PROTHALLIA AND SPOROPHYTES¹

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THE ADVENTITIOUS sprout of a fern prothallium is an outgrowth from a single cell, either inner or marginal, which develops in a manner similar to the outgrowth of a prothallial filament from a spore. Like a prothallial filament it produces an apical growing region, becomes heart-shaped, and may develop sex organs which are capable of functioning in fertilization.

Such outgrowths have been observed in fern prothallia under a variety of conditions. They were first observed on abortive prothallia of *Notochlaena*, *Allosorus*, and *Gymnogramme* by Hofmeister (1851). Since then they have been seen on old prothallia, arising chiefly from the basal regions, by Wigand (1854) on *Chrysodium crinitum*, Goebel (1877) on *Gymnogramme leptophylla*, DeBary (1878) on *Pteris cretica*, Bauke (1878) on *Aneimia* and Mohria, Goebel (1887) on *Osmunda regalis*, Campbell (1892) on *Osmunda claytonia* and *Osmunda cinnamomea*, Bruchmann (1904) on *Ophioglossum vulgatum*, Bruchmann

(1908) on *Lycopodium complanatum*, Pace (1910) on an unknown fern which resembles *Dryopteris*, and by Dopp (1927) on *Onoclea struthopteris*. They have been observed on prothallia growing very close together by Kny (1872) on *Osmunda regalis* and by DeBary (1878) on *Pteris cretica*; on prothallia that had been growing under weak light by Heim (1896) on *Lygodium japonicum*, *Alsophila australis*, *Balanium antarcticum*, and *Doodya caudata*, and by Stokey (1930) on *Alsophila exelsa*; on prothallia which had been grown under different wave lengths of light by Heim (1896) on *Lygodium*, *Alsophila*, *Balanium*, and *Doodya*, and by Klebs (1917) on *Pteris longifolia*. They have been induced in prothallia of *Gymnogramme chrysophylle* with moderate dosages of X-ray by Linsbauer (1926); on prothallia of *Alsophila australis* which had grown under a layer of water .8 cm. in thickness by Heim (1896); on prothallia that had been placed for a time in chambers containing an atmosphere of ether and chloroform by Heilbronn (1910) on *Asplenium*; on prothallia of *Aspidium*, *Balanium*, *Athyrium*, and *Ceratopteris* that had been plasmolyzed in different salt solutions and then deplasmolyzed by Isaburo Nagai (1914); and finally on prothallia that had been cut in various ways by Goebel (1905), Heim (1896), Linsbauer (1926a), and Albaum (1938).

Intact prothallia, grown under controlled conditions of light, temperature, and humidity, never give rise to adventitious outgrowths. The conditions described

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above under which they do arise are all abnormal. Since seed plants subjected to many of these abnormal conditions show a disturbance in the production or activity of their growth hormones, it was thought desirable to ascertain whether growth hormones are involved in the outgrowth of adventitious sprouts. More specifically, the experiments to be described were carried out with the following objectives: (1) to ascertain the conditions under which adventitious sprouts arise from cut pieces of mother prothallia; (2) to find out whether a synthetic growth hormone (3-indole acetic acid) either in distilled water solution or in lanoline paste can in any way control the outgrowth of these sprouts when applied to cut prothallia, and whether the applied hormone is transported through the cells in a polar fashion; (3) to ascertain whether the sporophyte plays any role in the outgrowth of adventitious sprouts from the fertilized prothallium.

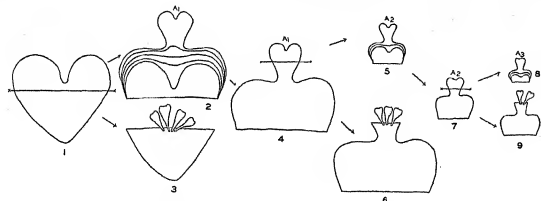


Fig. 1-9. Effect of successive removal of apical growing regions on the outgrowth of adventitious prothallia. Figures 2, 5 and 8 show the growth of the successively isolated apices. Figures 3, 6 and 9 show the polarized outgrowth of adventitious prothallia from the isolated basal pieces.

MATERIALS AND METHODS.—Prothallia of *Pteris longifolia* were grown from spores which were secured through the courtesy of Dr. Ralph C. Benedict of the Brooklyn Botanic Garden. Spore germination and isolation of experimental plants were carried out in the manner previously described (Albaum, 1938). After the prothallia were isolated from the germinating cultures, they were kept in a specially constructed incubator in which light, temperature, and humidity were maintained constant. All cuts, as before, were made with iridectomy scissors.

Data on growth were recorded by means of camera-lucida drawings, on the assumption that increase in surface area was a good index of the amount of growth at the end of any interval of time. The surface area of the camera-lucida drawings was measured with a polar planimeter (Keufel and Esser, no. 4240).

In the experiments which utilized growth hormone (3-indole acetic acid, Eastman Kodak Co.), the crystalline acid was dissolved in distilled water by slight heating. Where the growth hormone was applied to the plants in lanoline, the latter was first melted by heating, the crystalline auxin added, and the mixture well stirred. The growth hormone paste was applied to the prothallia by means of a pair of fine forceps.

Growth hormone from the prothallia was detected and assayed by means of the standard *Avena* technique (Went, 1928) and from primary leaves of the sporophyte by the less satisfactory root inhibition method (Lane, 1936).

RESULTS AND DISCUSSION.—Cutting experiments.—When a prothallium of *Pteris longifolia* is separated into apical and basal portions by a simple transverse cut (fig. 1), the apical portion undergoes a series of changes in form shown diagrammatically in figure 2. These changes have been described in detail elsewhere (Albaum, 1938). The old apical notch grows out and begins to regenerate a heart-shaped form by means of cell division and cell enlargement. In the absence of injury and under optimal conditions of light, temperature, and humidity, such an apical half never produces adventitious outgrowths. The basal portion, however, gives rise to one or more of these outgrowths. These tend to be polarized in their

origin, appearing, when the cut is made just behind the apical notch, near the cut surface and close to the center (fig. 3).

When the regenerated apex, designated as A_1 , is now cut as shown in figure 4, so that the actively dividing apical region is separated from its basal portion, the former once more undergoes a series of changes in form (fig. 5). The basal portion gives rise to a number of adventitious outgrowths similar to those described above. These, again, are polarized in their origin (fig. 6).

The new apical region, A_2 , may now be cut as shown in figure 7, and a regenerated apex, A_3 , will be formed (fig. 8). Adventitious prothallia arise from the basal piece (fig. 9). These experiments show that as long as the actively dividing apex is attached to the basal portion, no adventitious outgrowths appear from the latter. Tentatively, the assumption may be made that an actively dividing apex is producing something which has the ability to inhibit the outgrowth of adventitious prothallia.

How much of the apex must be present in order to bring about inhibition? To find an answer to this question, the cuts shown in figure 10 were made. All that remained in the notch region was a group of about seven elongated cells, the central one pre-

sumably being the apical cell. The behavior of the isolated wings is shown in figures 11 and 12. Each wing continues to grow at its inner surface and eventually regenerates the typical heart-shaped form of the prothallium. The growth curves of such wings are similar to those previously described for halves of isolated apices (Albaum, 1938). The behavior of the basal portion which has the remnant of the notch is shown in figure 13. It is apparent that the remnant of the notch region has grown out and regenerated the heart-shaped structure of the prothallium. When the heart-shaped form with the actively dividing, apical region is present, neither the regenerated basal portion nor the regenerated wings produce adventitious outgrowths, proving again that the apical regions are in some way inhibiting the outgrowth of adventitious prothallia.

This is taken to mean that the stimulus sent out by the apical regions to inhibit adventitious outgrowth is not transmitted through dead cells. There is another possibility. If one assumes that the inhibiting substance is chemical in nature, it may be diffusing out of the dead cells onto the filter paper so rapidly that not enough of it is getting through the dead cells to bring about the inhibitory effect.

If a wedge-shaped cut is made on either side of a prothallium, leaving a junction of living cells connecting the apex to the base (fig. 16), the apical tissue above the cut continues to grow, while no adventitious sprouts appear on the basal portion (fig. 17). In one typical experiment twenty-five prothallia were cut as shown in figure 16. In eighteen of these the cells forming the junction between apex and base remained alive, and no adventitious out-

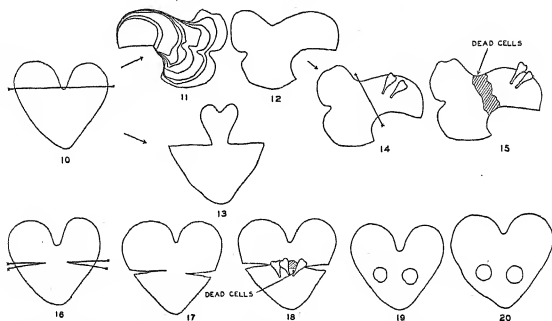


Fig. 10-20.—Fig. 10-13. Effect of partial removal of apical region on the outgrowth of adventitious prothallia. Figures 11 and 12 show the behavior of the isolated wings; figure 13, outgrowth of the remainder of the notch region with consequent inhibition of adventitious prothallia.—Fig. 14. Effect of removal of actively growing region of a wing on the outgrowth of adventitious prothallia from the remainder of the wing.—Fig. 15. Effect of a junction of dead cells between the actively growing region of a wing and more lateral portions on the outgrowth of adventitious prothallia.—Fig. 16-18. Effect of incomplete transverse cuts on the outgrowth of adventitious prothallia. In figures 16 and 17 a living junction of cells is present; no adventitious outgrowths. In figure 18 there is a junction of dead cells; adventitious outgrowths are produced.—Fig. 19-20. Effect of removal of internal regions of prothallium on adventitious outgrowth.

If either of the regenerated wings is cut as shown in figure 14, the apical heart-shaped portion continues to grow, while the isolated fragment produces adventitious outgrowths. Often it is not necessary to isolate the growing portion of a wing from its more lateral regions in order to bring about adventitious outgrowth. In a large group of experiments one finds that in a small number of the experimental plants a mass of cells between the growing region and the rest of the wing dies. That the cells are dead is evidenced by the fact that they have lost their chlorophyll and most of their cell contents. There is a continuity between the growing cells and the remainder of the wing through the almost empty cells with their cellulose walls; yet adventitious sprouts appear behind the dead cells just as they did when the growing region was removed by cutting (fig. 15).

growths appeared. In the other seven, however, the cells forming the junction died. Adventitious outgrowths arose behind the dead cells (fig. 18), indicating again that the inhibitory stimulus sent out by the apex cannot pass the dead cells or pass over in sufficient concentration to produce an inhibitory effect. One other type of cutting experiment was performed. By means of a capillary glass tube, several holes were cut out of intact prothallia (fig. 19). After several days the apical region continued to grow, but no adventitious outgrowths appeared behind the holes (fig. 20), showing again that as long as there is a continuity of living cells, the dominance of the apical regions is complete.

The cutting experiments described above show conclusively that under controlled conditions, where there is a junction of living cells between apical growing

regions and more basal ones, adventitious sprouts never appear. The absence of an apex and a non-living junction promote the outgrowth of adventitious prothallia.

Application of growth hormone.—What does the apex possess which enables it to exercise this inhibition? In a series of recent experiments Kaiser and Albaum (unpublished data), using a chloroform extraction method (Thimann, 1934), were able to extract from the apical regions of prothallia of *Pteris longifolia* a growth hormone or auxin. It has long been known that the terminal bud of seed plants inhibits the outgrowth of lateral buds lower down on the stem. The removal of the terminal bud permits the outgrowth of the lateral buds. Thimann and Skoog (1933, 1934) working on *Vicia faba*, showed that the terminal bud is the most active center for the production of growth hormone in the plant. This growth hormone could be obtained by diffusion from the tip, indicating that the latter transports the hormone basally. Removing the terminal bud and substituting for it an agar block containing growth hormone led to a continued inhibition of the lateral buds. Growth hormone, then, either directly or indirectly is able to inhibit the outgrowth of lateral buds of seed plants.

The fact that a growth hormone or auxin may be extracted by means of chloroform from the apical regions of the prothallium does not prove that such a hormone is being transported basally, since the chloroform extraction method, according to Went and Thimann (1937), is able to remove not only free transportable hormone, but also bound hormone. The latter is active in the *Avena* test. The first experiments were carried out, therefore, to ascertain whether the hormone in the apical regions of *Pteris longifolia* is transported through the cells of the apex. This was done by placing apical halves of large prothallia directly on *Avena* test plants, making sure of contact by placing some 10 per cent gelatin at the junction of the cut surface of the half and the test plant. Of six plants, clear-cut responses with curvatures ranging from 5.0° to 12.0° resulted in five, showing that the apical regions produce hormone which is carried basally.

That 3-indole acetic acid (heteroauxin) has many of the physiological properties of the auxins which have been isolated from seed plants is well known. The first experiments to ascertain whether growth hormone could inhibit adventitious sprouts were performed with solutions of 3-indole acetic acid made up in distilled water. Two concentrations were used: 10mg/l and 100mg/l. Basal halves of fern prothallia (fig. 21) were placed into Petri dishes containing each of these solutions on filter paper. The cut surfaces of the halves were placed in contact with the solutions. A number of bases were used as controls in water. The results at the end of 14 days are shown diagrammatically in figures 22 and 23 and in detail in table 1. 3-Indole acetic acid in the concentrations specified inhibits the outgrowth of adventitious prothallia almost completely.

TABLE 1. Effect of distilled water solutions of 3-indole acetic acid on the outgrowth of adventitious prothallia (see fig. 22, 23).

Number of plants	Average number of adventitious prothallia at the end of 14 days		
	Water control	10 mg./l.	100 mg./l.
20	2.0		
23		0.48	
14			0.24

The use of auxin solutions with small prothallia in the manner outlined is rather crude. With relatively large basal halves, the solution may not remain in contact with the cut surfaces, while small halves are usually surrounded completely with the solution. Pastes of lanoline containing growth hormone were, therefore, substituted. Of considerable advantage is the fact that the paste may be applied locally. In addition, the growth hormone is taken up from the paste very slowly so that the supply of hormone does not have to be renewed over a long period of time. The paste contained a concentration of 10 mg of the indole acid per gram of lanoline. The results of a typical experiment are shown in figures 24 and 25 and table 2. It is at once apparent that the lanoline paste containing the growth hormone almost completely inhibits the formation of adventitious outgrowths, just as in the case of the solution. Plain lanoline may have a slight inhibiting effect by itself; this, however, is by no means certain.

TABLE 2. Effect of 3-indole acetic acid in lanoline paste (10 mg./gram of lanoline) on the outgrowth of adventitious prothallia (see fig. 24, 25).

Number of plants	Average number of adventitious prothallia at the end of 7 days		
	No lanoline	Plain lanoline	Lanoline and growth hormone
4	6.8		
9		5.5	
23			0.17

There may be a possibility that the growth hormone in the paste is actually proving toxic to the prothallial bases. In order to rule out this possibility, the growth substance paste was allowed to remain on the plant for a time and then cut away (fig. 25). Shortly afterward, adventitious sprouts appeared behind the cut surface (fig. 26, table 3). The experiment just described is significant in another way. The lanoline containing growth hormone, when applied, behaves just like an apex in inhibition; as soon as it is cut away, just as with the living apex, inhibition is released.

Researches on seed plants have shown that when physiological concentrations of the hormone are used, transport is polar (Went, 1928; Beyer, 1928). With concentrations which are considerably greater than

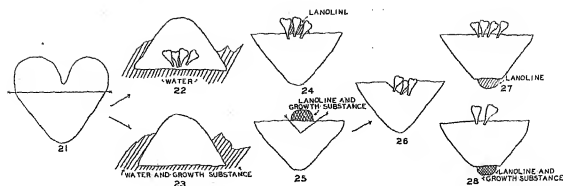


Fig. 21-28. Effect of growth hormone application on the outgrowth of adventitious prothallia.—Fig. 22-23. Inhibition of adventitious sprouts by distilled water solutions of 3-indole acetic acid (table 1).—Fig. 24-25. Inhibition of adventitious sprouts by 3-indole acetic acid in lanoline paste (table 2).—Fig. 26. Effect of removal of growth hormone paste on the outgrowth of adventitious prothallia (table 3).—Fig. 27-28. Effect of basal application of 3-indole acetic acid in lanoline paste on the outgrowth of adventitious prothallia (table 4).

those normally found in the plant, transport may take place in an upward direction; the latter type of transport takes place chiefly in the transpiration stream (Went and Thimann, 1937) in the same way that nutrient salts and water are conducted upwards. In the fern prothallium there is no specialized conducting system; conduction apparently takes place from cell to cell. Growth hormone paste was, therefore, placed at increasing distances back of the apical cut surface in order to find out whether inhibition of outgrowths occurs. The sketch (fig. 28) merely shows the paste applied at the basal cut surface; the paste was actually applied at varying distances from the apical to the basal cut surface. The results are presented in figures 27 and 28 and table 4. When the paste is applied anywhere back of the cut surface,

(Goebel, 1887). The writer has seen prothallia of this kind which have been growing for more than five years. A possible explanation of the way in which such abnormal prothallia may arise has been offered elsewhere (Albaum, 1938). If fertilization takes place, a sporophyte develops from the fertilized egg (fig. 32). The young sporophyte typically possesses a foot for attachment, a root, short stem, and primary leaf. For a time following fertilization the prothallium grows at the same rate as the normal unfertilized prothallium (fig. 32, table 5). Soon, however, the prothallium ceases growing (table 5), while the sporophyte, chiefly in the region of the primary leaf, begins to grow rapidly. With the cessation of growth in the prothallium, the normally small cells of the old apical region enlarge (Allen, 1914).

TABLE 3. Effect of temporary application of 3-indole acetic acid in lanoline paste (10 mg./gram of lanoline) on the outgrowth of adventitious prothallia (see fig. 25, 26).

Number of plants	Average number of adventitious prothallia			
	7 days after original cutting		7 days after paste removal	
	Plain lanoline	Lanoline and growth hormone	Plain lanoline	Lanoline and growth hormone
16	6.4		6.4	
17		0.59		3.2

the inhibition is reduced considerably. Because of the variability of the material, it is difficult to say whether the difference between the plain lanoline control and the lanoline-containing growth hormone is significant. Some of the growth hormone, however, might have diffused apically either through the cells or in a water film on the surface of the cells. Essentially, however, just as in *Avena*, transport is polar, in spite of the fact that high concentrations of the hormone were used. This may be ascribed to the absence of non-living conducting elements which might draw the hormone up.

Sporophytic inhibition.—If a fern prothallium is not fertilized, it continues to grow normally (fig. 29, 30, 31). After a long period of time, however, it loses its heart-shaped form and becomes ribbon-like

If a sporophyte-bearing prothallium is cut as shown in figure 33, so that the old apex is isolated from the basal part to which the sporophyte remains attached, the apical portion now produces adventitious outgrowths (fig. 34). The base with the sporophyte attached does not produce such outgrowths (fig. 35). If, however, the entire sporophyte is removed, the basal portion sends forth numerous adventitious outgrowths. Apparently the apex of the original prothallium has given over its role of hormone production to the sporophyte, which now has the ability to inhibit the outgrowth of adventitious sprouts. So long as the sporophyte is present and growing actively, adventitious outgrowths do not appear. Since the organ which is growing most rapidly in the young sporophyte is the primary leaf, it seemed likely that

the latter was the new center for growth hormone production. A number of primary leaves were removed and extracted with chloroform, and the extract was tested on *Avena* roots (Lane, 1936). An extract of 30 young primary leaves produced an inhibition of 42 per cent in the length of the test roots, indicating that the former probably contained considerable quantities of a growth hormone.

taining 10 mg 3-indole acetic acid per gram of lanoline. The results are shown graphically in figure 38 and 39 and in detail in table 6. It is apparent that the hormone completely inhibits the outgrowth of other leaves. In another series of experiments, the blade of the primary leaf was removed as before. After four days, a second leaf had made its appearance; this in turn was removed in the same way.

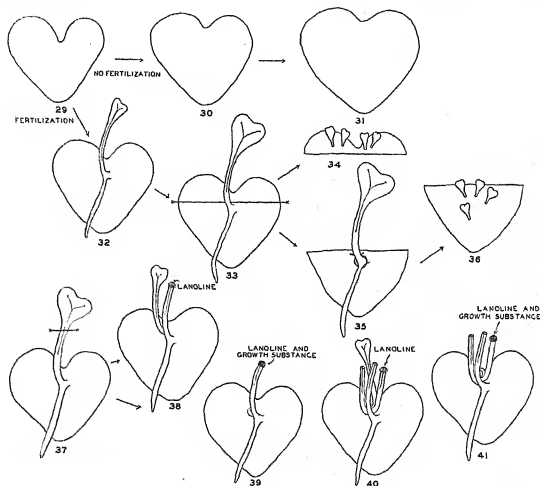


Fig. 29-41.—Fig. 29-31. Growth of unfertilized prothallia (table 5).—Fig. 32-33. Effect of growth of sporophyte on the growth of the prothallium (table 5).—Fig. 34-36. Effect of sporophyte on the outgrowth of adventitious prothallia.—Fig. 34. Growth of adventitious prothallia from an isolated apex of a sporophyte bearing prothallium.—Fig. 35. Inhibition of adventitious outgrowths from a basal portion of a prothallium by a sporophyte.—Fig. 36. Growth of adventitious sprouts from the basal portion of a prothallium following removal of the sporophyte.—Fig. 37-41. Inhibition of other leaves and adventitious sprouts by the application of 3-indole acetic acid in lanoline paste. Detailed data for figures 37-39 are shown in table 6.

If the primary leaf of a young sporophyte is the center for hormone production in the plant, and if the primary leaf is removed, one might expect to release the inhibition and promote the outgrowth of adventitious prothallia. In order to find out whether this is true, the green blade-like portion of the primary leaf was removed (fig. 37). After four days a second leaf appeared from a region which corresponded to the young stem and began to grow rapidly; no adventitious outgrowths appeared. The primary leaf was inhibiting not only the outgrowth of adventitious sprouts from the original prothallium, but also the outgrowth of other leaves. The question arose: Might the latter effect also be due to the growth hormone which the primary leaf produced? To find an answer to this question, a series of primary leaves was removed as described above (fig. 37). To the leaf stumps of one group of plants plain lanoline was applied; to another group, lanoline paste con-

After six days a third leaf had appeared and was growing rapidly. The third leaf was then removed, and upon the leaf stumps of some of the plants, lanoline containing growth hormone was applied; to the remainder of the plants, plain lanoline was applied. Ten days later those plants on which plain lanoline had been applied had given rise to a fourth leaf. On the plants of the other group no new leaf was present (fig. 40, 41). The lanoline containing growth hormone was removed from the plants of the latter group by cutting. From the plants of the former group the fourth leaf was removed. After seven more days the inhibited plants had produced their fourth leaf, while the untreated ones had given rise to a fifth leaf. During this entire interval most control plants had one primary leaf; in a few cases, they had developed two.

General discussion.— Previous experiments have shown that in the growth of a prothallium or of parts

of it which possess meristematic cells, the important factor determining the amount of growth attained after any interval of time is the total mass of tissue present and not the amount of the meristematic region (Albaum, 1938). The more basal regions apparently contribute materials to more apical regions, and these materials are used in the growth of the apex. It is probable that these materials are not raw

tions do not have to be absorbed from the environment. The idea that more posterior regions contribute materials to anterior growing regions is by no means a new one. It is Goebel's (1905) concept of an "attraction center." According to this idea more active apical regions draw on materials from less active basal regions and use them in growth. If one is to judge by growth activity, the apical

TABLE 4. Effect of apical and basal application of 3-indole acetic acid in lanoline paste (10 mg./gram of lanoline) on the outgrowth of adventitious prothallia (see fig. 24, 25, 27, 28).

Number of plants	Average number of adventitious prothallia at the end of 7 days			
	Plain lanoline apically	Plain lanoline basally	Lanoline with growth hormone apically	Lanoline with growth hormone basally
16	5.75			
20		7.0		
19			0.42	
19				3.7

materials absorbed from the environment but synthetic ones built up by the basal cells. The reason for this belief is that rapid growth is not dependent upon the presence of rhizoids through which the greater part of the absorption of raw materials (water and minerals) is carried on in an intact prothallium. When an apical portion of the same size as another intact prothallium is isolated by cutting, this apical portion lacks rhizoids and does not develop new ones for several days; yet the amount of growth is the same for the cut piece and the intact prothallium. This observation indicates that the materials which the basal portions supply to the apical growing por-

region of the fern prothallium is certainly the most active one. Evidence has been obtained which indicates that this is the region, too, which produces growth hormones having the ability to inhibit the outgrowth of the adventitious prothallia. As the prothallia grow, they produce larger amounts of growth hormone (Kaiser and Albaum, unpublished data). As they grow, they also apparently receive larger amounts of synthetic material from more basal regions. Some relationship appears to exist between the supply of synthetic material and the production of the growth hormone. Either synthetic materials from the basal regions bring about meristematic activ-

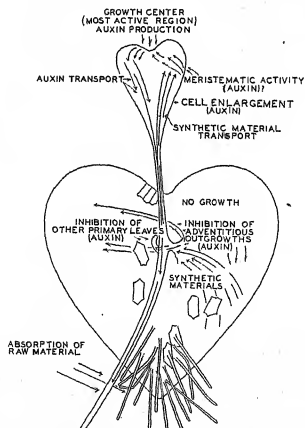
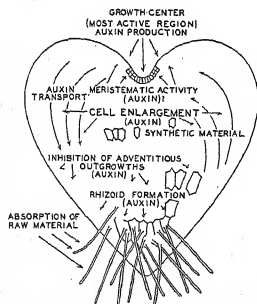


Fig. 42 (left). Hypothetical scheme showing role of growth hormones or auxins in the normal growth and development of a fern prothallium.

Fig. 43 (right). Hypothetical scheme showing role of growth hormones or auxins in the normal growth and development of a fern sporophyte.

ity which as a by-product produces more growth hormone, or the synthetic materials produce more growth hormone which secondarily stimulates mitotic activity and cell enlargement. That cell enlargement is stimulated by growth hormone has long been known; that mitotic activity is regulated by growth hormones is less universally accepted, although evidence for this view is rapidly being accumulated (see Went and Thimann, 1937).

the large amount normally coming from the apex inhibits their outgrowth, while the small amount resulting from isolation actually stimulates their outgrowth.

As pointed out in the introduction to this paper, different workers have shown that a diverse variety of conditions are capable of bringing about the formation of adventitious sprouts. Can the origin of the outgrowths in all these cases be explained in

TABLE 5. Growth of unfertilized and fertilized prothallia (see fig. 29, 30, 31, 32, 33).

	Area ($\times 4 \times 10^4 \mu^2$) at the end of			
	0 days	7 days	18 days	21 days
Average of 5 unfertilized prothallia ..	30.2	48.7	68.7	72.7
Average of 6 fertilized prothallia	32.9	49.2	52.9	52.9

One definite effect of growth hormone coming from the apex of the prothallium is the inhibition of the outgrowth of adventitious prothallia. This effect, as has been shown, is brought about by the polar transport of the hormone, chiefly through living cells. Another effect of the hormone, which has not been touched upon at all in this paper, is the induction of rhizoid formation. Some evidence has been obtained which indicates that the growth hormone plays a role in determining the formation of rhizoids (Kaiser and Albaum, unpublished). The ideas set forth in the last two paragraphs are summarized tentatively in the form of a diagram (fig. 42).

With the rapid development of the primary leaf of the sporophyte, the prothallium ceases growing. It appears that the synthetic materials formerly used by the apical regions of the prothallium in furthering their own growth are now transported to the primary leaf; as a result, the prothallium stops growing. One must assume that the primary leaf becomes more active in metabolism and growth than the old apex. The primary leaf now becomes the auxin producing center and one of the chief growth centers of the young sporophyte. The growth hormones produced by the leaf may bring about the enlargement of the leaf cells and perhaps promote cell division. As before, the growth hormones prevent the outgrowth of adventitious prothallia, and in addition they inhibit the outgrowth of all but the primary leaf. These ideas are summarized tentatively in figure 43.

It is difficult to say whether the production of adventitious outgrowths on the basal portions of cut prothallia occurs because the growth hormone supply is completely absent or because it falls below a certain level in concentration. This statement is prompted by a recent paper of Thimann (1937) in which he shows that roots, buds, and stems react to auxins in essentially the same way, except that they differ in their sensitivity to these substances, roots being most sensitive and shoots least sensitive. Each type of organ is stimulated by very low concentrations of applied hormone but is inhibited by sufficiently high concentrations. It may be that the outgrowth of adventitious prothallia is controlled in the same way;

terms of effects on the growth hormones contained in the prothallium? Such an explanation does seem to be possible. The appearance of outgrowths following cutting has already been discussed in detail. The appearance of adventitious outgrowths on old prothallia is probably due to a deficiency of hormone. Either the apex ceases to function, so that no hormone is sent back to bring about inhibition; or, in the case of long ribbon-like prothallia upon which outgrowths most often appear near the basal end, the hormone may be used up in cell enlargement and rhizoid formation, leaving too little to produce inhibition. In the case of prothallia growing close together, it has been observed that they are often, if not always, ameristematic and very small (Stokey, 1930). This would seem to indicate the inability to produce sufficient amounts of growth hormone to check the outgrowths. The development of outgrowths in weak light or in darkness may be correlated with the fact that growth substance can be produced only in the presence of light (Skoog, 1937).

TABLE 6. Effect of application of 3-indole acetic acid in lanoline paste (10 mg./gram of lanoline) to the primary leaf stump on the outgrowth of other leaves (see fig. 38, 39).

Number of plants	Number of second leaves at the end of 7 days	
	Plain lanoline	Lanoline and growth hormone
7	7	
9	9	
15		0
8		0

The production of adventitious outgrowths under different wave lengths of light, studied by Heim (1896) and Klebs (1917), may perhaps be explained by effects on the production and distribution of growth hormone.

The origin of outgrowths on prothallia treated with moderate dosages of X-ray may be due to one or more effects on growth hormones. In the first place, the hormone itself may have been inactivated. Skoog

(1935) has shown that in *Vicia faba* moderate dosages of X-ray (150–200R) inactivate the hormone both in the plant and in solution. Similar dosages also inhibit auxin production. The latter is correlated with the findings of Linsbauer (1926), who observed that with X-ray dosages similar to those employed by Skoog, cell division in fern prothallia was inhibited for a time.

In the case of adventitious outgrowths appearing on plants grown under a layer of water (Heim, 1896), it is quite possible that the available hormone diffused out of the cells, leaving a concentration insufficient to produce inhibition. With prothallia grown in an atmosphere of ether and chloroform, the appearance of outgrowths may have resulted from the well known action of ether in abolishing polar transport of hormone and in stopping all transport beyond that due directly to diffusion (van der Weij, 1934). Or, as Heilbronn (1910) has observed, the ether may have killed the meristematic cells, thus destroying the probable hormone producing center.

In the case of the appearance of adventitious prothallia following plasmolysis and deplasmolysis, Isaburo Nagai (1914) suggests that the plasmodesmata between cells are broken. Since it is quite possible that the hormone transport from cell to cell takes place chiefly through the plasmodesmata, the destruction of these might conceivably account for the appearance of the outgrowths.

SUMMARY

Adventitious outgrowths appear from cut pieces of prothallia of *Pteris longifolia* cultured under constant conditions of light, temperature, and humidity only when such pieces lack an actively growing meristematic region or when a junction of dead cells lies

between an actively growing region and more basal regions.

A growth hormone which may be extracted by chemical methods is transported from the apex through the cells of the prothallium to the base.

3-indole acetic acid, one of the synthetic auxins, when applied either in solution or in lanoline paste to isolated pieces of prothallia which normally give rise to adventitious outgrowths, has the ability to inhibit such outgrowths. Such an inhibition is most effective when the lanoline paste is applied through the apical cut surface.

Isolated apical regions from fertilized prothallia to which sporophytes are attached and have been growing for some time produce adventitious outgrowths. The basal portions which retain the sporophyte do not give rise to such outgrowths; upon removal of the sporophyte, however, the adventitious prothallia arise.

The auxin producing center of the young sporophyte is the primary leaf. The latter produces hormone which not only inhibits the outgrowth of adventitious processes from the prothallium, but also the outgrowth of other leaves. The role of the primary leaf in performing this function may be taken over by synthetic 3-indole acetic acid applied in lanoline.

The growth of the fern prothallium is discussed in terms of the growth hormone concept and Goebel's "attraction center" concept.

The appearance of adventitious outgrowths on fern prothallia under a variety of abnormal environmental conditions is discussed in terms of the growth hormone concept.

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INFLUENCE OF DEUTERIUM OXIDE ON PHOTOSYNTHESIS IN FLASHING AND IN CONTINUOUS LIGHT¹

Robertson Pratt and Sam F. Trelease

SOON AFTER deuterium oxide, or heavy water, became available in sufficient quantities for biological research, it was found that the rate of photosynthesis of *Chlorella* was only about 0.41 as rapid in buffers prepared with 99.9 per cent D_2O as in those prepared with H_2O (Curry and Trelease, 1935); CO_2 was supplied in excess, and light intensity was 6,500 lux (correction from 2,000). Subsequent tests, made with buffers prepared by mixing the two kinds of water, indicated that the three molecular species present in the mixtures— H_2O , HDO , and D_2O —were, to some extent at least, interchangeable in the photosynthetic process and influenced its rate independently of one another (Craig and Trelease, 1937). Since the influence of temperature on photosynthesis in the range of 10° – $30^{\circ}C$. was the same in 99.9 per cent D_2O as in H_2O , the same limiting reaction apparently determined the rate of photosynthesis in both kinds of water. With continuous light of high intensity—i.e., when the velocity of the dark-chemical reaction determined the rate of the total reaction—99.9 per cent D_2O strongly depressed the rate of photosynthesis. But with continuous light of low intensity—i.e., when the speed of the photochemical reaction determined the rate of the whole process— D_2O exerted but relatively little influence. This suggested that D_2O retarded the rate of the dark reaction, but had little, if any, effect on the photochemical stage of the process.

Intermittent illumination was used in the work here reported in order to obtain direct evidence concerning the influence of D_2O on the photochemical and dark reactions of photosynthesis. A preliminary account of one of the tests has appeared in *Science* (Pratt, Craig, and Trelease, 1937).

MATERIALS AND METHODS.—The D_2O was prepared in Professor Urey's laboratory by the method described by Brown and Daggett (1935), and it was purified in our laboratory by four successive distillations, as in previous experiments (Pratt, 1936; Craig and Trelease, 1937). The ordinary control water (H_2O containing 0.02 per cent D_2O), obtained from a laboratory still, was redistilled in the same manner as the D_2O .

Chlorella vulgaris was cultured according to the method previously described (Craig and Trelease, 1937). New stock cultures were always started daily. Cells used for inoculating new cultures and for photosynthesis measurements were separated from the culture solution and then were washed twice in distilled water to remove the adhering culture medium. This was accomplished by repeated centrifugation and decantation. Cells were taken only from four-day-old, rapidly growing cultures, which contained 6–7 million cells per cc. The populations were estimated from haemocytometer counts. New cultures were inoculated with approximately 35 million cells, giving an initial concentration of about 115 thousand cells per cc.

Cells to be used for photosynthesis measurements were soaked in non-buffered D_2O or H_2O for 30 minutes after the second rinsing with distilled water. They were then centrifuged, and the supernatant liquid was decanted. Following this treatment, the cells were suspended uniformly in 6 cc. of 0.1 M $KHCO_3$ prepared with 99 per cent D_2O or with H_2O , and 5 cc. of the suspension were transferred to the rectangular manometer vessel. Each vessel contained 25–35 million cells.

For measuring photosynthesis, the rate of O_2 evolution was determined by means of Warburg (1919)

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TABLE 1. Summary of calculations for intermittent illumination.

Duration of flash (sec.) ^a	Duration of dark interval (sec.)	Ratio dark interval ÷ flash	Flash + dark interval (sec.)	Flashes per min. total time	Sec. of light per min. total time	Percentage of light per min. total time	Min. of total time required for 1 min. actual illumination
.0045	.0122	2.71	.0167	3593	16.17	26.95	3.71
.0045	.0288	6.40	.0333	1802	8.11	13.52	7.40
.0045	.0622	13.82	.0667	900	4.05	6.75	14.81
.0051	.0858	16.82	.0909	660	3.37	5.62	17.80

^a Period during which light is available to 50 per cent or more of the cells.

manometers. CO₂ was supplied in excess by a 0.1 M solution of KHCO₃.² The temperature was 23.9 ± 0.05°C. All rates were corrected for respiration by tests in darkness.

The light system comprised a 500-watt projection lamp, a series of lenses, and a mirror to deflect the light beam on to the manometer vessels from below. The intensity of illumination at the bottoms of the vessels was measured by means of a Macbeth illuminometer and a Weston photoelectric-cell light meter, Model 603. The readings with the two instruments agreed well and ranged from 38,000 to 43,000 lux, depending upon the previous length of service of the lamp. Lamps were discarded (usually after about 25 hours) when they were no longer capable of yielding at least 38,000 lux at the bottoms of the vessels. Under the conditions of these experiments the rate of photosynthesis in continuous light with buffers made up with H₂O was independent of light intensity above approximately 18,000 lux. With D₂O buffers light saturation was attained with a considerably lower intensity (fig. 7, Craig and Trelease, 1937).

For obtaining intermittent illumination, a solid disk with suitable openings was rotated vertically in front of the light source. The duration of a flash was considered as being the time during which light was available to 50 per cent or more of the cells in the vessels as an opening in the disk passed across the light beam. The openings were so arranged that the duration of each flash was about 0.0045 second in the first three tests and 0.0051 second in the last. Each flash was followed by a relatively long dark interval—the length of which was varied from 2.71 to 16.82 times that of the flash. A summary of the calculations for intermittent illumination is given in table 1.

The vessels containing the suspension of *Chlorella* were shaken at a rate of 60–65 complete oscillations per minute. They were exposed to continuous light during an adaptation period of 15 minutes which permitted the vessels to come to the same temperature as the water bath. Manometer readings were then made at 3–5 minute intervals—first in continuous light for 25–35 minutes, then in intermittent illumination for a similar length of time, next in continuous light again, and finally in darkness (for the respiration

² According to Smith (1937), this solution contains about 1000×10^{-6} moles CO₂ per liter.

correction). The rate of photosynthesis was found to be the same in continuous illumination before and after exposure to flashing light.

The results of the experiments are given in tables 2 and 3, and they are plotted in figures 1–5.

RESULTS AND DISCUSSION. — Representative data from table 2 for an experiment with each of the four dark intervals employed are plotted in figure 1, with time in minutes as abscissas and c.mm. of O₂ evolved per 100 million cells as ordinates. The number near each curve gives the slope, or rate of photosynthesis. By examining the portions of the curves representing O₂ evolution in intermittent light, it may be seen that with a short dark period following each flash of light (fig. 1A) the slope of the curve for photosynthesis in H₂O is much steeper than that for photosynthesis in D₂O. But as the length of the dark period is extended (fig. 1B, 1C, 1D), the curves tend to become parallel. With the two longest dark intervals (fig. 1C, 1D), the slope of the curve for D₂O is essentially the same as that for H₂O. The amount of variation among the different experiments may be seen in table 2.

The averaged results of all the tests are shown in figure 2, in which the rate of photosynthesis per minute of total time elapsed is plotted against the length of the dark period between flashes (average data from table 3). It is apparent that with the two longest dark periods the curve for D₂O nearly coincides with that for H₂O. The results shown graphically in figures 1 and 2 are interpreted to mean that when the dark period after each light exposure was long enough to allow the dark reaction to proceed to completion in D₂O, the rate of photosynthesis was as great in D₂O as in H₂O. The experiments with intermittent light therefore furnish direct evidence for the conclusion that D₂O retards the rate of the dark reaction of photosynthesis without appreciably affecting the photochemical stage of the process.

This conclusion is supported by the data for the ratio of the rate of photosynthesis in D₂O to the rate of this process in H₂O. These are shown in the fifth column of table 3. With continuous light of high intensity the ratio had an average value of 0.403.³ But with intermittent light the magnitude of this

³ This is in good agreement with the average value 0.41 previously reported (Curry and Trelease, 1935; Craig and Trelease, 1937).

TABLE 2. *Photosynthesis in Chlorella vulgaris*. CO_2 supplied by 0.1 M KHCO_3 . Light intensity, 38,000–43,000 lux. Temperature, 23.9°C. Cells from 4-day-old cultures.

Duration of dark interval (sec.)	Duration of flash (sec.)	Sec. per min. light available to 50% or more of cells	Intermittent light		Continuous light ^b	
			C.mm. O_2 liberated per 100 million cells per min. of total time		C.mm. O_2 liberated per 100 million cells per min. of total time	
			H_2O	D_2O	H_2O	D_2O
.0122	.0045	16.17 = 26.95%	4.32	2.56	6.83	3.09 ^a
			7.45	4.00	9.99	4.37
			6.71	1.70	7.98 ^a	2.56
			av. 6.16	av. 2.75	av. 8.27	av. 3.34
.0288	.0045	8.11 = 13.52%	2.72	1.50	6.42	2.69
			2.26	2.17	4.89	2.98
			3.52	2.14	7.61	3.10 ^a
			2.60	2.20	8.11	3.51
			4.00	1.30	7.82	2.06
			3.66		9.63	
			2.96		8.25	
			3.69		7.52	
			4.00		7.90	
			3.06		6.96 ^a	
			av. 3.25	av. 1.86	av. 7.51	av. 2.87
.0622	.0045	4.05 = 6.75%	1.44	1.78	6.12	3.72
			1.54	1.60	7.04	4.06
			1.31	1.30	7.44	2.85
			1.64	1.63	8.77 ^a	3.37 ^a
			1.71	1.72	9.07	3.88
			1.21		6.57	
			1.68		8.15	
			1.87		9.22	
			1.80		6.90	
			2.03		9.23	
.0858	.0051	3.37 = 5.62%	av. 1.62	av. 1.61	av. 7.85	av. 3.58
			1.22	0.90	9.05	2.58
			1.36	1.71	7.54 ^a	4.45
			1.78	1.64	9.20	3.58
				0.77		2.14
				1.82		3.23 ^a
			av. 1.45	av. 1.37	av. 8.60	av. 3.20
					av. 8.06	av. 3.25

^a Data from this experiment are plotted in figure 1.^b Averages of data obtained before and after each test with intermittent light.

ratio increased until it approximated unity when the dark period between light flashes was sufficiently long (0.0622 second or longer). The results of these tests therefore all point to the conclusion that the principal influence of D_2O on photosynthesis was to retard the dark-chemical (or Blackman) reaction and that D_2O exerted little, if any, influence on the photochemical stage of the process.

Figure 3 shows the rate of photosynthesis per minute of total time (table 3) plotted against the percentage of time that light was available. It may

be seen that with a reduction in the amount of light received by the cells per minute, photosynthesis was reduced relatively more in H_2O than in D_2O . It is also evident from figure 3 that in neither H_2O nor D_2O was photosynthesis in intermittent light reduced commensurately with the amount of illumination. Therefore, per unit amount of light supplied, the amount of photosynthesis in intermittent light was greater than that in continuous light.

Figure 4, plotted from the data of table 3, indicates photosynthetic yields per flash of light. The

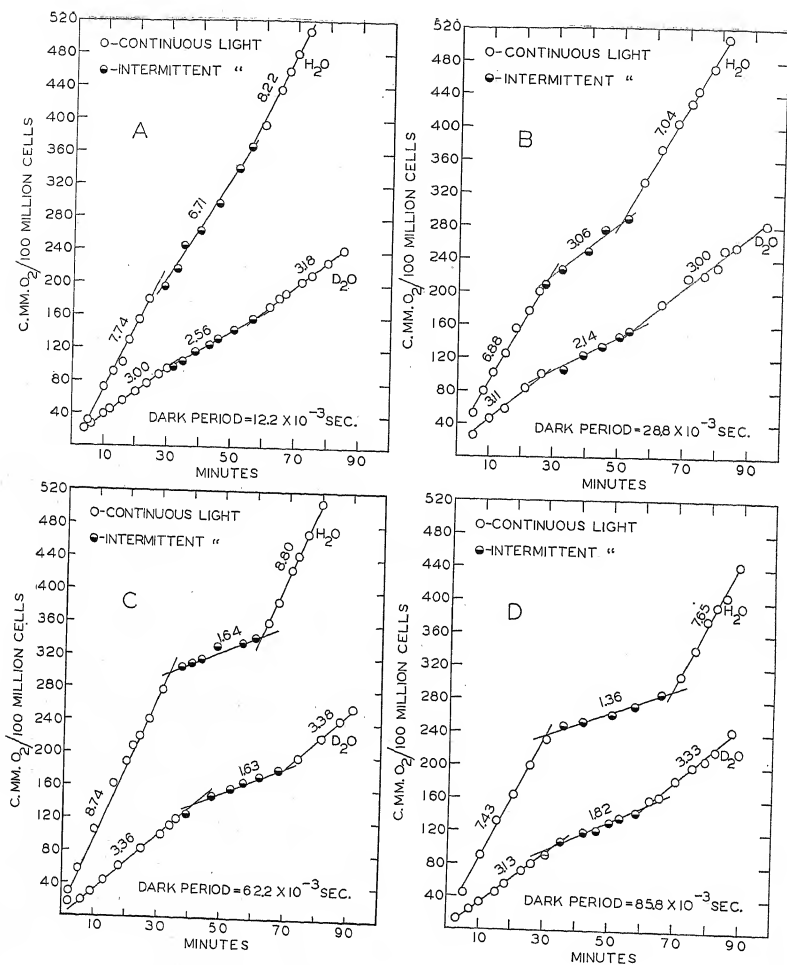


TABLE 3. Summary of photosynthesis data for *Chlorella vulgaris*. CO_2 supplied by 0.1 M KHCO_3 . Light intensity, 38,000–43,000 lux. Temperature, 23.9°C.

Duration of dark interval (sec.)	Min. of total time required to yield 1 min. of actual illumination	C.mm. O_2 liberated per 100 million cells per minute of total time			C.mm. O_2 liberated per 100 million cells per min. of actual illumination			
					Absolute		Relative	
		H_2O	D_2O	$\text{D}_2\text{O}/\text{H}_2\text{O}$ ^a	H_2O	D_2O	H_2O	D_2O
0	1.00	8.06	3.25	.403	8.06	3.25	1.00	1.00
.0122	3.71	6.16	2.75	.447	22.85	10.20	2.84	3.14
.0288	7.40	3.25	1.86	.572	24.05	13.76	2.98	4.23
.0622	14.81	1.62	1.61	.994	23.99	23.84	2.98	7.34
.0858	17.80	1.45	1.37	.945	25.81	24.39	3.21	7.50

^a Ratio of the rate in D_2O to that in H_2O .

ordinates represent oxygen evolution per minute of actual illumination (not total time elapsed), and abscissas represent the length of the dark intervals between flashes. The ordinates are proportional to yields per flash of light, since there were 13,333 flashes per minute of actual illumination. These graphs show that as the length of the dark period after each flash was increased, the amount of photosynthesis per

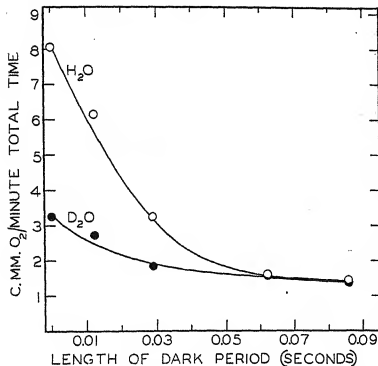


FIG. 2. Rate of photosynthesis of *Chlorella vulgaris* per minute of total time as a function of the length of the dark period.

flash increased to a certain maximum value and then remained constant. Since the two curves reach approximately the same maximum, it is evident that when the dark period after each flash was long enough to obtain maximum yield, the amount of photosynthesis per flash was the same with D_2O as with H_2O .

It may be seen, however, that the curve for H_2O reaches its maximum level earlier than the one for D_2O . This is in accord with expectations based on the hypothesis that D_2O influences only the dark reaction of photosynthesis. The points on the abscissa scale at which the curves in figure 4 become hori-

zontal may be considered as representing approximately the time required for the completion of the dark reaction after exposure for 0.0045 second to white light having an intensity of 38,000–43,000 lux.⁴ Since the rate for D_2O in continuous light is about 0.41 of that for H_2O , one would expect the dark reaction to require approximately 2.4 times as long to proceed to completion in D_2O as in H_2O if the chief influence of D_2O is to retard the dark reaction of photosynthesis. It is interesting to note, therefore, that the D_2O curve becomes horizontal at approximately 0.0622 second, while the H_2O curve becomes horizontal at about 0.0288 second. The former value (0.0622) is 2.2 times as great as the value for H_2O . The agreement between expected and actual values may be considered reasonably close if the variation among the different tests is taken into account.

Figure 5 is similar to figure 4 except that the amounts of photosynthesis per minute of actual illumination are plotted as relative values, the amount in continuous light being expressed as unity (data from last two columns of table 3). Per unit amount of light supplied, photosynthesis in H_2O was about 3.0 times as great in intermittent illumination as in continuous illumination. With D_2O , as would be expected, intermittent illumination was about 7.5 times as effective as continuous light. Since the methods employed in these experiments differed somewhat from those of other investigators (Emerson and Arnold, 1932a, 1932b; Warburg, 1919), the values given here cannot be compared with the data published by these authors for photosynthesis in H_2O .

The results of the various experiments performed in this laboratory indicate (1) that the rate of photosynthesis in 99–100 per cent D_2O is about 0.41 of that in H_2O when continuous white light and CO_2 are furnished in excess at approximately 25°C. (Curry and Trelease, 1935); (2) that the value of this ratio decreases approximately semi-logarithmically with an increase in the initial fraction by volume of D_2O ; (3) that D_2O and H_2O probably participate in the same stage of the photosynthetic process, but (4)

⁴ The amount of dark-chemical reaction that is completed during each flash has been neglected in these experiments.

that they react independently of each other (Craig and Trelease, 1937). Tests with various intensities of continuous light also indicate (5) that D_2O influences principally the dark reaction in photosynthesis. The present experiments with intermittent light furnish direct evidence for this conclusion and seem to leave little doubt that D_2O retards the velocity of the dark-chemical reaction in photosynthesis without appreciably influencing the photochemical stage of the process.

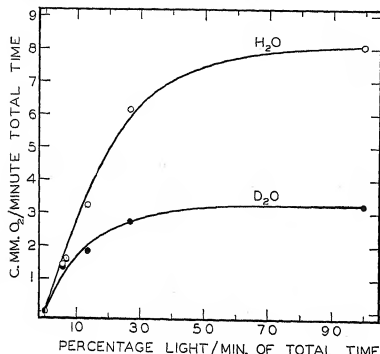


Fig. 3. Rate of photosynthesis of *Chlorella vulgaris* per minute of total time as a function of the percentage of time that light was available to 50 per cent or more of the cells.

The data presented in this paper might be interpreted as indicating that D_2O merely has a narcotic effect, like that of HCN and other poisons, on the dark reaction. This explanation, though not entirely eliminated, seems improbable, since previous experiments (Craig and Trelease, 1937) furnished evidence that H_2O and D_2O are, to some extent at least, interchangeable in the photosynthetic process.

There have been numerous attempts to analyze the process of photosynthesis, step by step, and several hypothetical formulations of the photosynthetic mechanism have been published (Burk and Lineweaver, 1935; Emerson and Arnold, 1932a, 1932b; Warburg, 1926). In this connection, perhaps the most important implication of our experimental results is that H_2O as well as D_2O enters into the dark stage rather than into the photochemical stage of photosynthesis.

SUMMARY

Previous experiments showed that deuterium oxide, or heavy water, reduces the rate of photosynthesis of *Chlorella vulgaris* to about 41 per cent of that with ordinary water, and they indicated that the reduction is due to a retardation of the dark-chemical (or Blackman) reaction. The present experiments were designed to test this hypothesis.

Manometric determinations were made of O_2 evolution by *Chlorella vulgaris* suspended in 0.1 M $KHCO_3$ prepared with H_2O and with 99 per cent D_2O . The temperature was $23.9^\circ C$, and the light intensity was 38,000 to 43,000 lux at the bottoms of the manometer vessels. Photosynthesis was measured in continuous and in intermittent illumination. Each flash lasted approximately 0.0045 second and was followed by a relatively long dark period, the duration of which was varied from 2.7 to 16.8 times that of the flash.

With a sufficiently long dark interval between flashes the rate of photosynthesis in D_2O was the same as that in H_2O .

The maximum photosynthetic yield per flash of light was the same in D_2O as in H_2O . But to obtain maximum yield with D_2O , the dark period between flashes had to be more than twice as long as that required for maximum yield with H_2O .

Per unit amount of light, intermittent illumination gave about 3 times as much photosynthesis in H_2O as continuous illumination. In D_2O it gave about

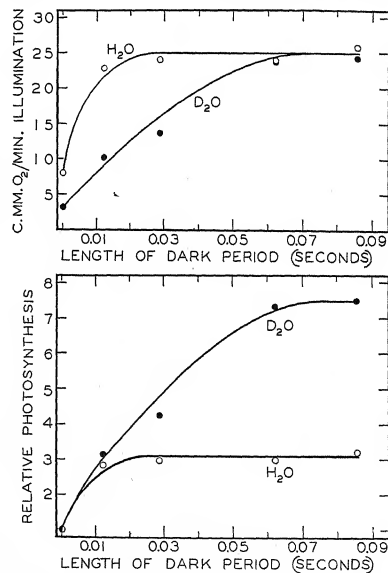


Fig. 4 (above). Rate of photosynthesis of *Chlorella vulgaris* per minute of actual illumination as a function of the length of the dark period. The values are proportional to the photosynthesis per flash.

Fig. 5 (below). Relative rate of photosynthesis of *Chlorella vulgaris* per minute of actual illumination as a function of the length of the dark period. The rate in continuous light is expressed as unity.

7.5 times as much photosynthesis as continuous illumination.

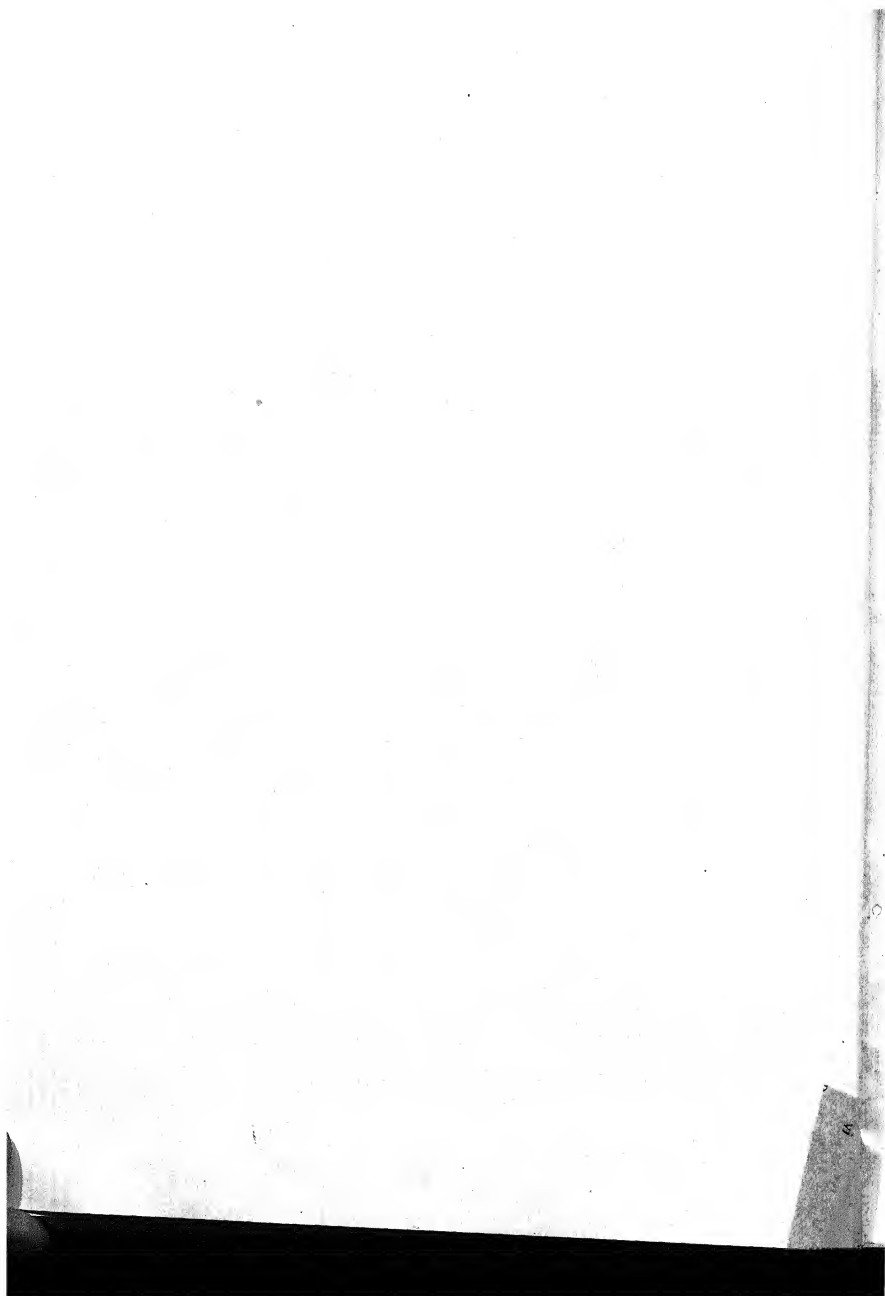
These experiments furnish direct evidence for the conclusion that D_2O retards the dark-chemical reaction of photosynthesis, but has little, if any, effect on the photochemical stage of the process.

An important implication of this work is that H_2O , as well as D_2O , enters into the dark-chemical reaction of photosynthesis.

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STRUCTURE AND DEVELOPMENT OF REGNELLIDIUM DIPHYLLUM¹

Duncan S. Johnson and M. A. Chrysler

Foreword

The late Professor Duncan S. Johnson, who for many years had been a student of the Marsileaceae, was during the last months of his life engaged in a study of *Regnellidium diphyllum*, Lindman, material for which he had been able to secure only after persistent efforts. Unfortunately that study was incomplete at the time of his death, although some of its results were then in the form of extended notes. It was at the request of Mrs. Johnson that I undertook to prepare the present paper from a study of Professor Johnson's material and notes. The part I have taken has indeed constituted a labor of love.

The sections on development of leaf and sporocarp represent almost wholly the work of Professor Johnson, while I am mainly responsible for the several sections on anatomy and for the discussion. It is planned to follow this paper by one on the development of sporangia and spores.—M. A. C.

SOURCES OF MATERIAL.—It was in 1892, on the first Regnell expedition, that Lindman discovered the plants which he accordingly named *Regnellidium*, a new genus of the Marsileaceae (Lindman, 1904). The place of collection was on the banks of the Rio Grande do Sul, in southern Brazil. The plant was not found again until November 1935, when it was located at Santa Maria by Dr. W. Rau and at Sao Leopoldo by Dr. J. Dutra. The two new stations are each about 250 kilometers from where Lindman found the plant forty years earlier, and they are about the same distance from each other. At the request of the first-named author of the present paper, these two Brazilian physicians kindly supplied him with fixed material and ripe living sporocarps from their collections, and he was at last enabled to undertake an anatomical and developmental comparison of *Regnellidium* with *Marsilea* and *Pilularia*. From some of those living sporocarps new plants were obtained, and these have been grown to maturity in the greenhouse of the Department of Botany of the Johns Hopkins University at Baltimore. They produced sporocarps late in 1937, and their culture is to be continued.

From the material collected by Lindman, two bits of stem bearing a few mature roots, leaves, and sporocarps were supplied for this study through the courtesy of the Naturhistoriska Riksmuseet, at Stockholm, and two sporocarps were supplied through the courtesy of the U. S. National Herbarium, in Washington.

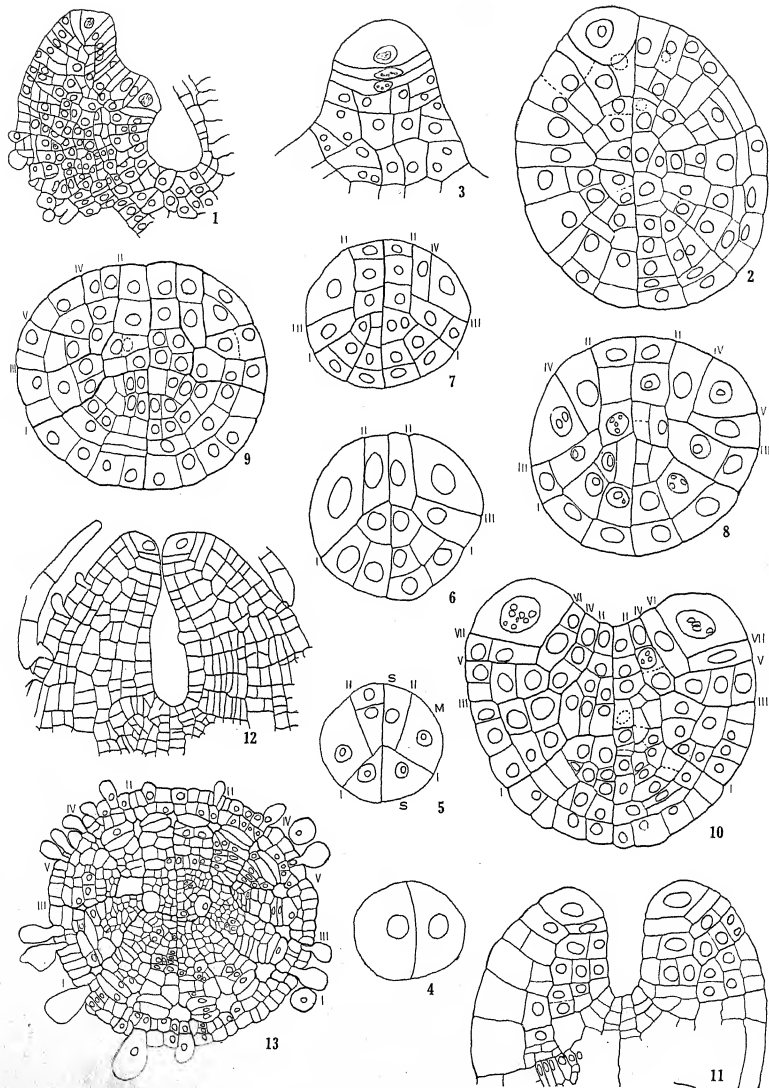
THE MATURE STEM.—The account by Lindman (1904) shows that *Regnellidium* like other Marsilea-

ceae is amphibious and that its sporocarps mature only when the plants are left stranded by fall of the water level. The submerged stem is creeping and relatively thick, the largest of several pieces studied being 3 mm. in diameter (Lindman says it may exceed 3 mm.). The mature internodes have a length of 4 to 10 times the diameter, as is shown in Lindman's original sketch of the terrestrial shoot. Internodes as well as nodes bear many adventitious roots, and when young both nodes and internodes bear numerous rusty brown several-celled trichomes. The stem branches freely, the branches apparently always arising laterally and at the base of a leaf as in other Marsileaceae. In the submersed stem the epidermis is a single layer of rectangular cells 28–40 μ wide, 135–250 μ long, and with their outer wall 1.5–2.0 μ thick. The guard cells of the infrequent stomata are of the type normal for Marsileaceae and range from 50 to 60 μ in length. The trichome-bearing cells are scattered rather regularly over the surface of the stem, about 25–30 per sq. mm. Each of these basal cells is shorter radially than the other epidermal cells, so that the stalk cell of the trichome sits in a depression. Each basal cell is about 12 μ square at base, 20 μ or more square at the outer end. It bears a funnel-shaped stalk cell which is frequently cutinized and in turn bears a 4–5-celled tapering trichome lying almost parallel to the surface, with its apex pointing forward on the organ and its basal projection directed backward on the stem (fig. 12, 22). The general structure of the trichome is more like that of *Marsilea* than that of *Pilularia globulifera* (cf. Meunier, 1888).

The cortex consists of three primary regions (fig. 27). There is an outer firm cortex 140–210 μ in thickness, made up of 3 or 4 (rarely of 2 or 5) layers of rather compact polyhedral cells from 15 to 60 μ in diameter. These have but few and small spaces between them and in some cases show numerous tannin cells 15–45 μ in diameter. It is this firm outer ring of the cortex that maintains the cylindrical form of the stem. A middle region of the cortex is occupied by 20–25 or more large longitudinal air canals 450–650 μ in radial dimension and 100–250 μ in tangential width. These are separated by one-layered partitions which are about 8–14 cells in radial width. These partitions are perforated by small intercellular spaces and are connected and supported laterally by one-layered transverse partitions 2–4 mm. apart, each composed of about 25 or 30 short-armed stellate cells that thus allow ready passage of gases lengthwise through the stem. The cell-walls of these partitions are evenly thickened on all sides to about 2 or 2.5 μ . The inner region of the cortex is made up of some 4–7 layers of polyhedral cells having the same range

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in size as those of the outer cortex. The innermost cells are tightly packed, while the outer cells of this region fit more loosely. Scattered through this region also may be seen tannin cells, although in some stems (e.g., those grown in the Johns Hopkins greenhouse) they may be practically absent.

Portions of the stem growing after the plants become stranded (fig. 39, 40) differ from the submersed portions in being a trifle over half as wide. Although the number of cell layers in the outer and inner cortical regions is about the same, the radial partitions are much shorter, showing only 4 or 5 cells in radial extent instead of the 8-14 found in the submersed stem. Hence the longitudinal canals are nearly circular in transverse section, and they occupy a relatively small portion of the emerged stem.

The central cylinder (fig. 28) is an amphiphloic solenostele, typical in all respects except that in accordance with the habitat the xylem shows reduction to a single (locally double) ring of vessels. The average diameter of the stele is about 700μ , and the thickness of the ring from external to internal endodermis is 180μ . As may be seen in figures 29 and 39, the leaf-traces break off from one edge of the foliar gap before becoming detached from the other edge, a feature common in horizontal fern rhizomes. The endodermal layer is continuous around each edge of a gap, and the cells show a Caspary's band occupying the whole extent of the radial walls. These cells take a vigorous stain with safranin. Inside the endodermis is a 2-3-layered pericycle, followed by a well-marked phloem in which the sieve tubes frequently reach a diameter of 50μ . Sieve tubes may be absent from portions of both outer and inner phloem. The vessels are remarkably round in outline, rather thick-walled for an aquatic, and show a conspicuous variation in diameter, a series of wide ones (about 70μ) being followed by a series of much narrower ones (17μ). Several small areas of protoxylem are visible. The vessels of the metaxylem are of the regular scalariform type.

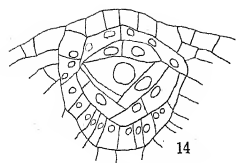
THE MATURE LEAF.—The leaf of *Regnellidium*, like that of *Marsilea*, varies greatly in size and also in character of surface, depending on whether the plant grows in air or in water. As described and figured by Lindman, the leaf has two pinnae instead of the

four found in *Marsilea*. These range from broadly wedge-shaped to nearly reniform. Lindman says that the petiole of the sterile (aquatic) leaf has a diameter of 1.7 to 2 mm. and that it may reach 20 cm. in length; the petiole of an aerial fertile leaf is often but half this length. Each leaflet of a sterile leaf may grow to 30 mm. or more in radial length and 40 mm. in breadth. Leaflets of a fertile (terrestrial) leaf are about 10×15 mm. The venation is strictly dichotomous, with no anastomoses except at the margin, in which region all the veins join up into a stout bundle parallel with the margin.

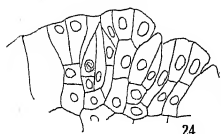
The petiole and pinnae of the young leaf are densely strigose, and in the emerged leaf these hairs are long persistent, being found on the outer face even of the fully matured and opened pinnae. In submersed leaves most trichomes have disappeared by the time the pinnae have unfolded. The persistent basal cells of the trichomes are to be found thickly scattered over the surface of the mature petiole and pinnae, being like the trichomes themselves most abundant on the outer side of the pinna.

In transverse section the petiole shows a single-layered epidermis, the cells of which are rectangular, about 200μ in length, 30μ in each of the other dimensions. The outer wall is only slightly thickened. Stomata are infrequent, and in the ones measured the guard cells are about 58μ long, while the pair are 31μ wide. The persistent basal cells of trichomes are numerous and have a diameter of about 20μ . The cortex of the petiole, like that of the stem, shows three concentric regions. The outer region about 100μ thick is of 2-4 layers of thin-walled isodiametric cells about 40μ across. The second concentric region is one of about 600μ radial thickness formed by generally 14 single-layered radial partitions that separate as many longitudinal air canals in the petiole. The individual cells in these partitions show a width of about 45μ in either direction. An occasional tannin cell is found in these partitions, 1 or 2 at most in the section of each partition. Perforations of the radial partitions in the petioles studied are less frequent than in those of the stem. The walls of all cells in these partitions are very thin. These long partitions are joined by widely perforated transverse partitions about 1-4 mm. apart longitudinally. The

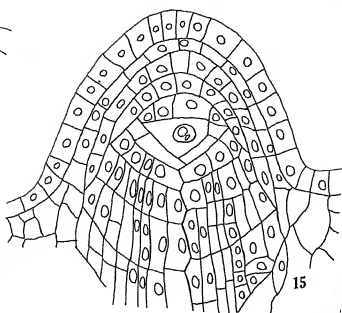
Fig. 1-13.—Fig. 1. Tip of young stem, with apical cell of leaf beginning to cut off segments at the right. Long. section. $\times 300$.—Fig. 2. Leaf initial cut out from a sector just to the left of the median line of the stem. Trans. section of stem at about the stage shown in fig. 1. $\times 400$.—Fig. 3. Apical cell of leaf cutting off segments. The plane of this section is at right angles to that of fig. 1—i.e., the stem visible at base of figure is cut transversely. $\times 400$.—Fig. 4. Trans. section of tip of leaf—i.e., in a plane parallel to surface of the stem, showing two segments and beginning of the median wall. $\times 400$.—Fig. 5. Slightly older leaf, trans. section. On each side of the median wall sector walls I and II leave a marginal cell between them. S, S, sectors; M, marginal cell. $\times 400$.—Fig. 6. Older stage, with three sectors cut off on each side. Sector I has divided by perichinal and antichinal walls. $\times 400$.—Fig. 7. Further divisions have taken place in the sectors, of which four are visible on each side. $\times 400$.—Fig. 8. Five sectors are present on each side; further divisions have taken place in the older sectors. $\times 400$.—Fig. 9. Formation of a perichinal wall in each marginal cell has ended the process of cutting off sectors, five of which are present on each side. $\times 400$.—Fig. 10. Beginning of formation of leaflets through continued activity of the marginal cells. Seven sector walls have been formed. $\times 400$.—Fig. 11. Older stage of developing leaflets. The marginal cells now function as apical cells. $\times 400$.—Fig. 12. Formation of leaflets and trichomes. $\times 400$.—Fig. 13. Trans. section through petiole at stage where air canals have appeared. The sector walls can still be made out, also the large vessel of each side of the young vascular bundle. $\times 300$.



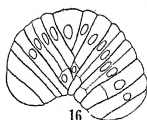
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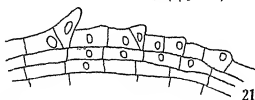
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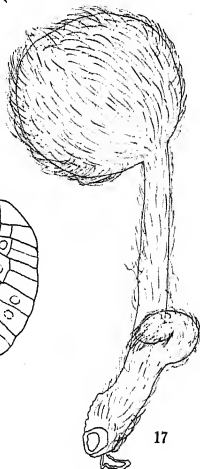
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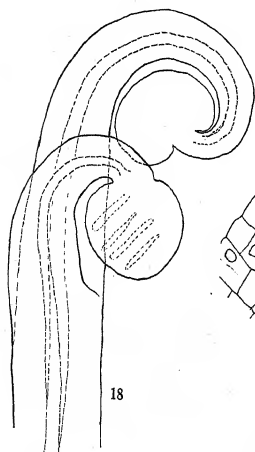
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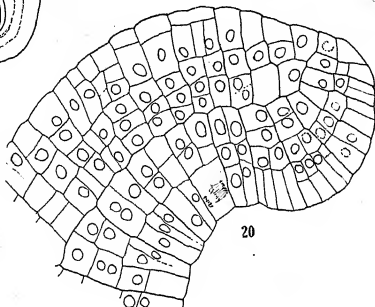
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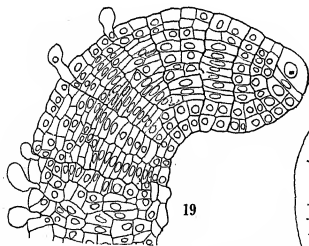
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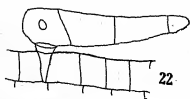
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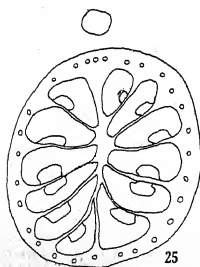
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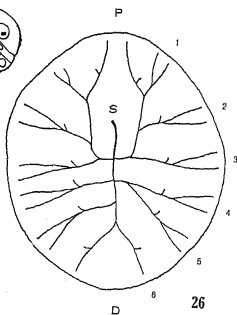
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inner cortical region is made up of 5 or 6 layers of thin-walled polyhedral cells 15–70 μ in diameter with intercellular spaces at almost every cell juncture. Characteristic of this region is the abundant supply of starch grains in all cells; five to ten tannin cells may also occur scattered in each 10 μ section.

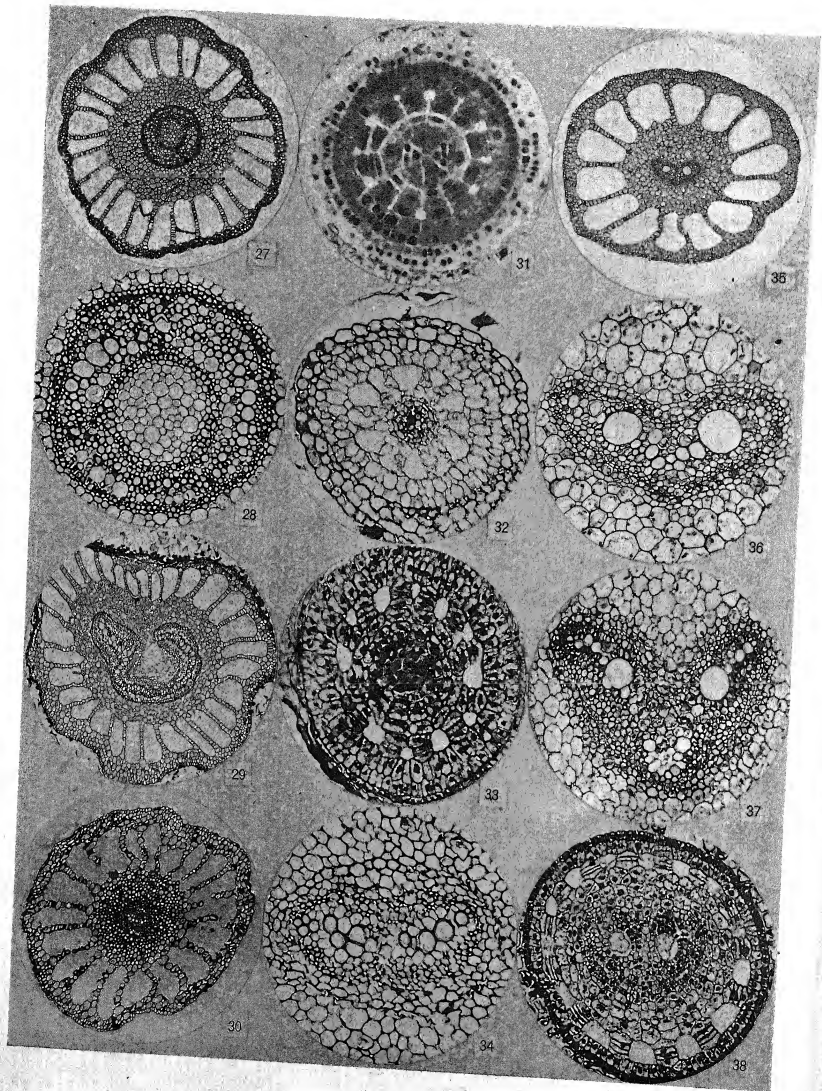
The endodermis surrounding the vascular bundle consists of small cells (about 13 μ in radial diameter) with walls devoid of noticeable thickening. In most of its course through the petiole the bundle is distinguishable by its bilateral structure and the presence of two relatively large vessels, one on each side of the middle line. These may reach a diameter of 0.1 mm. Other vessels or tracheids with diameter not over one-third as much adjoin the large ones so as to produce a flattened V-shaped mass of xylem (fig. 36), the inner or concave side of the V representing the adaxial side. Three groups of protoxylem are present, one at the angle of the V, and one at each extremity. The protoxylem groups are readily identified not only by the narrowness of the tracheids, but also by their lack of lignification in immature petioles. Phloem is present in limited amount on both sides of the xylem group. Toward the base of the petiole the V becomes more and more flattened until the xylem mass in transverse section is only slightly curved, and the marked variation in diameter of the tracheary elements is no longer in evidence. It is in this location that the bundle of the sporocarp branches off from the margin of the petiolar strand of a fertile leaf (fig. 40). Incidentally, this branching may be regarded as a case of dichotomy. Probably on account of the fact, mentioned earlier, that the leaf-trace breaks away from one side of its foliar gap while it is still attached to the opposite side (fig. 29, 39), the petiolar bundle lies with one edge toward the stem (fig. 40). The real adaxial side of the petiole can, however, be readily identified, for a series of sections show that the inner side of the V is continuous with the adaxial surface of the leaf-trace. It is evident, moreover, that the V-shaped bundle corresponds with the primitive C-shaped leaf-trace exhibited, for example, in *Todea*. Traced upward, the angle of the open V-shaped bundle decreases until less than 90° (fig. 37); meanwhile the two wide vessels taper off until they are no wider than the

other elements of the xylem. The angular region of the V broadens out, and from it two processes arise, giving the bundle the form of "H" (fig. 51), all four extremities of which rapidly extend as the two pinnae appear. Soon the joining bar in the H disappears, so that the vascular supply at the region of separation of the pinnae consists of two parallel much elongated concentric bundles. Each of these immediately begins the process of forking; this process gives rise to two parallel rows of bundles (fig. 49), each of these numbering about 16 at the level of separation of the pinnae. The phloem on the adaxial side, never abundant, disappears, leaving each bundle collateral. The radially arranged canals of the cortical region of the petiole are continuous with the ventral air chambers of the blade (fig. 50). Stomata are present on both surfaces, and the cuticle is poorly developed. The mesophyll is lacunar throughout, showing no palisade layer nor mechanical elements.

DEVELOPMENT OF THE LEAF.—The leaf of *Regnelledium* arises from a two-sided initial having somewhat the form of a bi-convex lens. The edges of this initial face up and down the length of the horizontal stem (fig. 1). The successive leaf initials arise on the upper surface of the stem, close to the midline of this surface, and alternately to the right and left of this line (fig. 2). The exact manner of cutting out the leaf initial from a segment of the stem initial was not determined, but in several cases it was evidently cut out of a sector of about one-quarter of one of the segments of the bifacial stem initial.

Each segment cut off from the leaf initial—"primary marginal cell" of Hanstein (1862) and Sadebeck (1874)—is approximately semicircular (fig. 4). Its radial width is about three or four times its thickness, and it is often slightly thicker at the outer margin and is of course somewhat concave toward the initial. As the segment grows older, it flattens out pretty completely to assume the form of the half of a plane disk lying transverse to the leaf-axis. The total number of segments formed by a leaf initial is twenty-odd. The first wall appearing in each segment of the leaf initial is a longitudinal and nearly radial anticline (fig. 5). It starts from just ventral to the middle of the median wall and curves backward slightly to meet the outer cylindrical wall at

Fig. 14–26.—Fig. 14. Early stage in development of an adventitious root. Three of the four cutting faces of the apical cell are visible, and the two outer layers of the stem are beginning to bulge. $\times 400$.—Fig. 15. Older stage in development of root. The cells of the plerome are especially plain. Three layers of root-cap have pushed outward the two outer layers of the stem. $\times 400$.—Fig. 16. Tip region of young leaf cut obliquely and showing activity of the apical cell distal to the two leaflets. $\times 300$.—Fig. 17. Habit drawing to show relations of leaflets, petiole and sporocarp. $\times 5$.—Fig. 18. Diagram showing curvature of stalk of leaf and of sporocarp at stage when soral canals are beginning to appear. $\times 19$. Young leaf, axial section; apical cell still active. $\times 200$.—Fig. 20. Young leaf, section taken to one side of axial plane so as to show beginning of formation of a leaflet. (cf. fig. 44). $\times 225$.—Fig. 21. Early stages in development of trichome. $\times 400$.—Fig. 22. A nearly mature trichome. $\times 400$.—Fig. 23. Young soral initial, not yet imbedded. $\times 400$.—Fig. 24. Anticline section through young sorus such as appears in fig. 52, showing two soral initials and between them the wall cells which elongate radially so as to imbed the sori. $\times 400$.—Fig. 25. Diagram of horizontal section through capsule, to show position of the sori on partitions between soral canals. S, position of stalk of sporocarp. $\times 6$.—Fig. 26. Diagram of vascular system of capsule, viewed from near point of attachment of stalk, S. P, proximal and D, distal ends of capsule. The girdling bundles of one side are numbered 1–6, and from each a short branch arises, supplying a sorus; the bundle then forks and the branches so formed run to the ventral side of the capsule. $\times 6$.



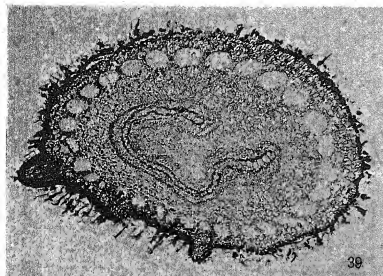
from one-third to two-fifths the way around from the posterior edge of the median wall. This "wall I" thus cuts out a sector of one-third to two-fifths of the semicircular segment of the leaf initial. This and the three or four succeeding longitudinal anticlines in each segment may therefore be known as "sector walls." The portion of the segment left after cutting off sector I may be known as a marginal cell of the second grade. Each later sector wall is more or less parallel either to the median wall or to the second preceding sector wall (fig. 6 et seq.). Thus the second sector wall (II, fig. 6) is nearly parallel to the median wall and runs from sector wall I to the ventral face of the young leaf somewhat to one side of the median wall. The portion of the segment left between sector wall I and sector wall II is a marginal cell of the third grade. Sector III is cut off from the dorsal side of the tertiary marginal cell by sector wall III which runs from sector wall II to the outer, cylindrical wall of the segment and is nearly parallel to sector wall I (fig. 8). Sector wall IV is very nearly parallel to wall II. Sector wall V (the last to be formed) is cut off toward the dorsal side from the marginal cell of the fifth grade and is therefore approximately parallel to sector wall III (fig. 8, 9).

In the twenty-odd segments of the leaf initial that are to form the petiole of the leaf only five sectors are cut out of each segment. Then a pericline appears in the marginal cell of the fifth grade, which event ends its activity as a marginal cell (fig. 9). The further division of each sector of the segment of the leaf initial proceeds with striking regularity at least through the first steps and results at first in cutting out the layer that corresponds to the vascular bundle (plerome), another equivalent to its cortex (periblem), and a third layer to its epidermis (dermatogen). In sector I the first wall formed is a pericline, at about half way from center to surface (fig. 6). This is soon followed by a (radial) anticline cutting the outer cell in two, and this by a pericline in each of the outer cells. This pericline separates the epidermis with its stomata and trichomes and an outer layer of cortex outside the air canals from the inner cortex with its partitions between the air canals. The central triangular cell cut off by the first pericline in sector I is divided by further anticlines to form a dozen or more cells which enter into the construction of the dorsal portion of the vascular bundle of the petiole. Sector II is first divided, about the time the sector wall III appears, by a pericline that cuts off its inner third or half (fig. 7). A second pericline outside the first separates an outer cell destined to form epidermis and one or more layers of

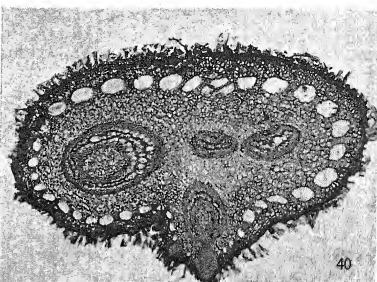
outer cortical tissue of partitions between air canals from an inner cell that divides so as to form twenty-odd cells of vascular tissue and several layers of inner cortex (fig. 7, 8). The inner cell, cut off by the first pericline in sector II, divides to quarters by two longitudinal walls at right angles (fig. 9). Of these quarters the one farthest from the median wall and from the ventral surface gives rise by growth in transverse and longitudinal directions and *without further division in either plane* to the one large vessel in each half of the vascular bundle of the petiole (fig. 13, 38). The remaining three-quarters of the inner third of sector II becomes divided by many longitudinal and frequent transverse walls to form various xylem and phloem elements of the ventral half of the vascular bundle (fig. 13). The inner end (one-third or two-fifths) of sector III is cut off by a pericline and later by several longitudinal anticlines to contribute about 20 or 30 cells to the lateral portion of the vascular bundle—i.e., to the right of the large vessel in the right half of the petiole or to the left of that in the left half (fig. 9, 13). The middle portion of sector III is devoted to the formation of inner layers of the cortex, about the vascular bundle, while an outer third or thereabouts gives rise to the epidermis, to an outer layer of cortex lying between the epidermis and the air canals, and to the longitudinal and transverse partitions between the air canals (fig. 13, 38). Sector IV may possibly form a few vascular elements at its inner end, though this was not determined with certainty. Its outer portion behaves essentially like that of sector III. The whole of sector V and the whole of the marginal cell left after sector V is formed are also cut by successive periclinal cells that serve to delimit inner and outer cortex and epidermis. Neither of these in the petiole participates in the formation of the vascular bundle (fig. 13).

The development of the air canals in the petiole is initiated by the separation of cell walls of the cortex from each other along the median wall, along each of the sector walls, and along the primary radial anticline in sector I (fig. 9, 13). With the increase in diameter of the petiole the epidermis increases in circumference and remains continuous, as is true of all the tissues from the endodermis inward and for several layers of cortical cells without this. But the cortical cells from two or three layers outside the endodermis out to the fourteen longitudinal air canals are rounded off from each other, leaving considerable air spaces interconnected with each other in all directions (fig. 36). Out beyond these layers of inner cortex the cells separate widely tangentially to leave only radial plates of cells to form the fourteen radial

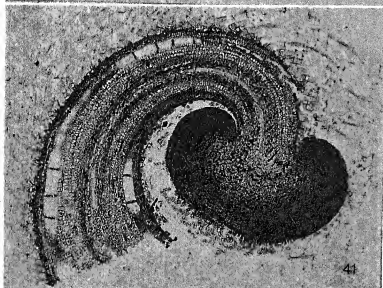
Fig. 27-38.—Fig. 27. Submersed stem, trans. sec. $\times 20$.—Fig. 28. Central cylinder of submersed stem. $\times 75$.—Fig. 29. Submersed stem at region of a leaf gap. $\times 30$.—Fig. 30. Stalk of sporocarp. $\times 35$.—Fig. 31. Young root, showing origin of the 12 air canals. $\times 135$.—Fig. 32. Nearly mature root. $\times 100$.—Fig. 33. Half-grown root, central cylinder still immature. $\times 125$.—Fig. 34. Vascular bundle of stalk of sporophyll. $\times 160$.—Fig. 35. Petiole, middle region. $\times 15$.—Fig. 36. Vascular bundle of petiole, showing the two large vessels. $\times 75$.—Fig. 37. Vascular bundle of immature petiole, upper region. $\times 90$.—Fig. 38. Half-grown petiole, with the two large vessels and developing air canals. $\times 90$.



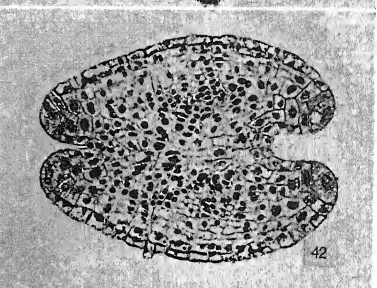
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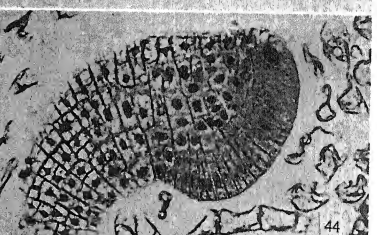
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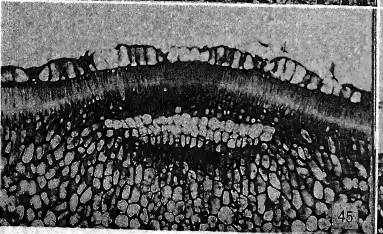
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single-layered perforated partitions that separate the longitudinal air canals (fig. 35) and also the occasional still more freely perforated transverse partitions that join and help support the radial ones (fig. 41).

In the development of the single dorsiventral vascular bundle of the petiole it is clear from the study of longitudinal sections of successively older leaves that the quarter of the inner third of sector II that forms the one large vessel of each side of the vascular bundle is not divided by either longitudinal or transverse walls. This is clearly true, though at least four mitoses may occur within its protoplast, since a longitudinal section through a still circinate coiled petiole (fig. 41) may show as many as fourteen nuclei in a single tracheal element (fig. 43). The total number of nuclei that may finally be formed in a single protoplast may be considerably greater, since the young vessel shown in figure 43 not only showed as yet no indication of degeneration of the protoplast but the scalariform thickening of the walls had not yet begun to form. The number of longitudinal and transverse cell divisions that may occur in other cells derived from the same segment of the initial cell of the leaf is very considerable, as is evident from comparison of the young vessel with the cells surrounding it in figures 41 and 43. The developmental history of this one cell is evidently unique among all the derivatives of each segment of the leaf initial of *Regnellidium*, as it was earlier shown to be in the leaf of *Marsilea* (Johnson, 1898).

Beyond the twenty or more segments of the leaf initial that go to make up the petiole are four or five additional segments on each side that go to form the single pinna of that side (fig. 44). Beyond these are at least two segments on each side that do not participate in the development of the pinna (fig. 16). These might perhaps be considered homologous with the segments of the leaf initial that in *Marsilea* give rise to the upper or distal pinnae. It is thus evident that the leaf initial of the sterile leaf of *Regnellidium* must cut off altogether some 30 to 35 segments that go to make up the whole leaf—i.e., the petiole, the two pinnae, and the short tip beyond the pinnae.

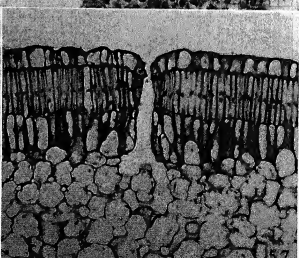
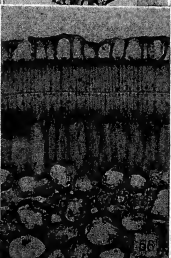
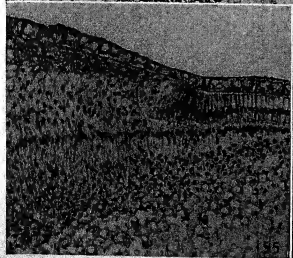
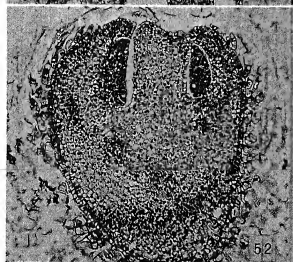
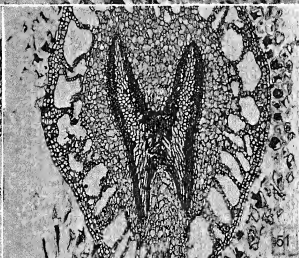
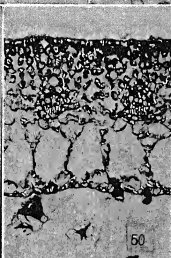
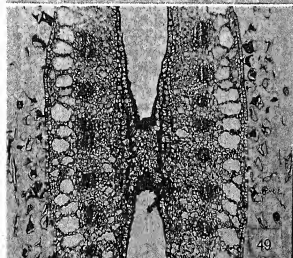
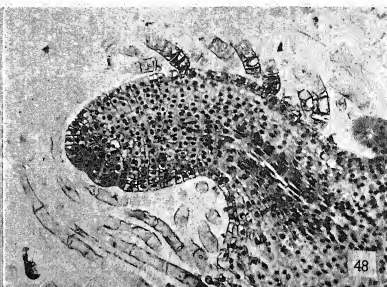
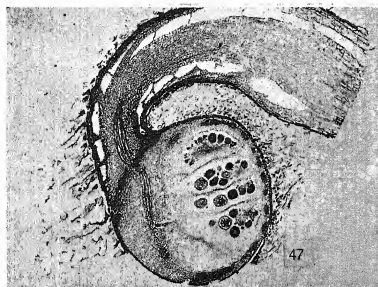
The pinna of the leaf of *Regnellidium* is developed by the continued activity, in three or four segments of the leaf initial, of the marginal cell of the fifth grade, which instead of having its outer portion cut off by a pericline to form an epidermal and one or two cortical layers of cells, continues cutting off "sectors" alternately toward dorsal and ventral sides (fig. 10-12). The total number of these sectors formed in the segments devoted to the development of the

lamina has not been determined, but it must be some dozens since the pinna is barely distinguishable when sectors VI and VII have been formed, and six or eight sectors can be counted in one-tenth the radius of a pinna that has not yet reached one-twentieth its mature size. It is evident from a comparison of a median sagittal section of a young leaf (fig. 19) with sections at one side of, but parallel to, this, that the apical cell of the leaf still persists and that at least three or four of the earlier segments of this initial have participated in the formation of the pinna. This is a feature that was pointed out in describing the development of the leaf of *Marsilea* (Johnson, 1898, p. 126). From the time that the marginal cells have cut off sectors VI and VII, they begin also to expand tangentially in the plane of the lamina and parallel to the margin of the leaf, so that the lamina finally comes to have the form of a folding fan that is widely opened (fig. 41). This rapid tangential expansion is accomplished by the tangential growth and through the multiplication of these marginal cells by frequently appearing radial anticlines in marginal cells themselves as well as in the recently formed sectors (fig. 44). With this fan-like expansion of the pinna as a whole, there is repeated forking of the procumbent strands which are to form the dichotomous vascular system of the blade.

The outer layer of cells of each surface of the leaf blade of *Regnellidium* is cut off by periclinal near the surface of each sector cut from the marginal cell. This outer layer becomes differentiated gradually into the stomata, trichomes, and ordinary epidermal cells of the upper and lower sides of the mature blade. These epidermal cells become more and more wavy in outline as they are farther from the base of the pinna.

Trichome initials make their appearance when the leaf is not more than 0.3 mm. in length. Each trichome arises from a small cell cut off by a slanting wall from that part of an epidermal cell which lies toward the tip of the leaf (fig. 12, 21). The trichome initial protrudes as a bulbous process which is soon cut off from the basal portion by a cross-wall. A stalk cell is next cut off, and this takes the form of a short funnel, the outer edge of which is at an early date developed as a brownish cutinized rim. Meanwhile the outer cell elongates nearly parallel to the surface of the leaf and chiefly in the distal direction, and several cross-walls appear. Finally the tip develops as a lash, while a short backwardly directed process makes its appearance (v. description of the mature trichome under section on stem).

Fig. 39-46.—Fig. 39. Emerged stem, trans. sec. The leaf trace is still attached to one edge of the gap. $\times 35$.—Fig. 40. Orienting photograph—emerged stem just above level of exit of leaf-trace. At the left, the central cylinder; below, a branch and root-anlage; at the right a leaf-trace from which has just branched the smaller bundle which supplies a sporocarp. $\times 35$.—Fig. 41. Half-grown leaf, nearly median section, showing air canals, one of the large vessels, the fan-shaped blade. $\times 35$.—Fig. 42. Young pinnae at level of junction with petiole. The apical cells are still active. $\times 195$.—Fig. 43. One unit of the large vessel shown in fig. 41. Fourteen nuclei are visible. $\times 195$.—Fig. 44. Very young leaf, section a little to one side of median line (cf. fig. 20), showing origin of a pinna. $\times 225$.—Fig. 45. Trans. sec. of dorsal wall of capsule, just distal to attachment of stalk, showing epidermis, hypodermis and the island of brown-walled cells. $\times 95$.—Fig. 46. Sagittal section through dorsal wall of capsule at attachment of stalk, showing the "overlap" with the two hypodermal layers separated (cf. fig. 55). $\times 50$.



THE ROOT.—The roots of the mature *Regnellidium*, all adventive, are but little branched and have a diameter of 0.4 to 0.6 mm. The epidermal or piliferous layer consists of somewhat elongated cells 20–30 μ wide, many of which give rise to root hairs. The cortex of the root like that of the stem shows three primary regions (fig. 32). The outer three-layered portion just beneath the epidermis is of compact isodiametrical cells a trifle smaller than those of the epidermis. Beneath this outer cortex is a series of 12 longitudinal air canals separated by perforated radial partitions one cell thick and four (three to five) cells in radial extent, which in turn are supported by transverse partitions consisting of stellate cells. The inner cortical region is made up of one or two layers of polyhedral cells of about the same range of size as the outer region. The innermost layer of the cortex is developed as endodermis, which is frequently a conspicuous feature on account of the strong thickening of its brown walls. The cells of this layer are flat elliptical in form, and the thickening when present occurs throughout the walls. Occasional cells remain unthickened, constituting the so-called "transfusion cells."

The stele has a diameter of about 80 μ , and its xylem is of the diarch type so common among ferns. Several of the centrally located vessels are larger than the other elements of the metaxylem. The characteristic thickening of the walls is scalariform. Upon each flank of the xylem mass is a small amount of phloem, and the whole is surrounded by a single layer of unspecialized pericycle.

DEVELOPMENT OF THE ROOT.—The root initials arise on the ventral side of the stem and are covered by two cell layers (the epidermis and outermost layer of cortex) which are pushed outward by growth of the young root. These layers, especially the epidermal one, retain their power of growth for a time and form the outer part of the root-cap. They acquire rather thick walls and stain intensely with safranin. The root initial early acquires the form of a tetrahedron with its base parallel to the surface of the stem and is easily distinguished by its rich protoplasmic nature. As it enlarges, segments are cut off parallel to the inner sloping faces and to a smaller number parallel to the outer face (fig. 14)—that is, it has four cutting faces, as is characteristic of ferns. The cells cut off by the periclinal walls build up as

usual the root cap, while from the inner segments are cut off the cells which promptly form dermatogen, periblem, and plerome (fig. 15). In transverse section the young root for some distance from the apex bears imprint of the three-sided origin of its tissues (fig. 31). Thus three primary triangular divisions can readily be recognized, each of which is twice divided radially in the outer portions. In the middle cortical region the radial rows (of four cells each) split apart, giving rise to the twelve radial canals which are seen in the mature root.

THE MATURE SPOROCARP.—As may be seen from Lindman's figure, a single sporocarp arises from near the base of a petiole, and the stalk is recurved near its junction with the capsule, especially in the young condition. The capsule is nearly spherical in form, measuring 6–8 mm. in length, while the width and height are 5–7 mm.; its surface is covered with stiff hairs which are appressed to the surface as in the case of the hairs borne on the stem. At the point of insertion of the stalk on the capsule, the former is continued for a short distance as a raphe, but no tubercles are present. The capsule thus presents a number of features which are in contrast to those of *Marsilea*. Internally the capsule is divided in the sagittal direction by a well-defined partition, so that it is plainly bilateral. On each side of this partition are six (occasionally five) soral canals extending parallel to the sagittal plane in the dorsi-ventral direction. These canals are separated from each other by delicate septa which extend in a more or less radiating direction from the central partition (fig. 25). Upon these septa the receptacles of the sori form strong ridges extending in the dorsiventral direction. Since there are on each side six sori and five septa, it is obvious that on one of the septa there is a sorus attached on each side. It is found, however, that each sorus has its own vascular supply arising, as will be seen later, from one of the so-called girdling bundles.

The capsule wall, as may be seen from figure 57, presents several well-defined layers. The epidermis consists of almost cubical cells about 35 μ in diameter interrupted at intervals by the basal cells of the trichomes and by guard cells of the stomata. The walls of this layer are strongly lignified. This is followed by a remarkable layer (fig. 56, 57) which was observed in *Marsilea* as early as 1846 by Mettenius

Fig. 47–57.—Fig. 47. Young sporocarp, sagittal section, showing the soral canals and sori, also the S-shaped crook in the vascular bundle of the stalk. $\times 20$.—Fig. 48. Very young sporocarp, nearly sagittal section. The soral initials are beginning to develop, and the curvature of the stalk is as yet slight. $\times 150$.—Fig. 49. Middle portion of pinnae from nearly mature leaf, at point of separation. The vascular bundles are forking. $\times 35$.—Fig. 50. Mature blade, with vascular bundles, air canals and trichomes. $\times 110$.—Fig. 51. Petiole a short distance below the level of fig. 49. Compare fig. 37. $\times 30$.—Fig. 52. Developing capsule, trans. sec., showing sori, soral canals still connected with the exterior by pores. $\times 75$.—Fig. 53. Very young leaf, section taken through region of curvature and showing origin of both pinnae. $\times 35$.—Fig. 54. Long. section through stem tip with leaf angle at left. At the upper side of the latter a sporocarp is just making its appearance. Compare fig. 1. $\times 200$.—Fig. 55. Sagittal section through wall of capsule just distal to attachment of stalk. The "overlap" of the two layers of hypodermis appears in this limited area. Compare fig. 46. $\times 55$.—Fig. 56. Trans. section through dorsal wall of mature capsule showing epidermis, outer hypodermis (prismatic layer) with the refractive line, inner hypodermis, inner part of wall. $\times 125$.—Fig. 57. Wall of nearly mature capsule showing the same layers as fig. 56, also a stoma. The prismatic layer is still undifferentiated. $\times 105$.

and thoroughly discussed by Russow (1872), who called it "Prismenschicht." The cells making up this layer are columnar (about 90μ high and 13μ wide), and their walls are greatly thickened, although not lignified. Near the middle of the cells the thickening is especially pronounced, taking the form of a constriction which almost divides each cell into an inner and an outer half. The sum of the constrictions of adjoining cells produces even under low magnification the appearance of a well-defined narrow line (fig. 56) dividing the layer in the periclinal direction. The third layer constitutes an inner prismatic layer, in which the cells, while palisade-like, are broader and much less uniform in radial length. Their walls are greatly thickened, but there is no median refractive line. This layer and the outer prismatic layer constitute a hypodermis. Following this is a zone consisting of rounded cells with plentiful intercellular spaces. The photograph (fig. 57) shows that these spaces communicate with the external atmosphere by means of passages piercing the columnar layers and opening by way of well-marked stomata. The guard cells are depressed slightly below the surface, but their walls are not greatly thickened. As many as ten stomata may be counted in a single section through a capsule, so that they are abundant to a rather unexpected extent. It would be interesting to establish a relation between the number of stomata per unit surface and the length of time during which a sporocarp remains alive in the different genera. The viable period in *Marsilea* is of course proverbial. Germination tests of capsules of *Regnellidium* probably two years old yielded negative results, which are not surprising in view of the excellent aeration provided by the abundant stomata.

Immediately distal to the region of attachment of stalk to capsule and in the median plane occurs the peculiar "overlap" of two layers of hypodermis (fig. 46, 55). The layer passing through the point of attachment appears to slip beneath one which is continuous around the distal and ventral regions of the capsule. As is shown in the photograph, the latter layer ends abruptly and is swollen and slightly curved toward the surface of the capsule. This overlap has been observed also in *Marsilea* and *Pilularia* (Johnson, 1933), but its significance appears problematical.

Almost immediately distal to the "overlap," in the median region and about 6 cell-layers below the surface, occurs a conspicuous group of cells (fig. 45) whose walls have a reddish-brown color. This group of cells extends about 600μ longitudinally and 350μ transversely, with a thickness of about 70μ . In this region the inner prismatic layer is not developed, and the outer prismatic layer is thinner than it is to right and left of the median region. The cells which make up this group are in 1-3 layers and have a rounded form or are slightly elongated perpendicular to the surface of the capsule. This group of cells no doubt corresponds to the one shown in Russow's figure representing the somewhat more specialized condition in *Marsilea*.

The vascular supply of the sporocarp arises a short distance above the level of origin of the leaf-trace from the stele. It has already been shown and is seen from figure 39 that the leaf-trace breaks off earlier from one edge of the leaf gap than from the other, and that the trace extends outward from the edge of the gap as a ribbon-shaped strand. Soon after the trace finally becomes free from the stele, the strand that supplies the sporocarp breaks off from that edge of the trace nearest the stele. These relations are made clear by the "orienting photograph," figure 40. It is thus seen that the vascular supply of the sporocarp is distinctly lateral with respect to the leaf. Unlike the petiolar bundle, the sporocarp strand does not acquire a pair of large vessels nor does it become V-shaped in section, but retains its flattened form. All the elements of both the xylem and the poorly marked phloem are narrow and crowded, and the strand is concentric. Toward the capsule the xylem becomes slightly curved (fig. 34), with the concavity adaxial, and three groups of protoxylem are evident, situated just as are the corresponding elements of the petiole. At the region of attachment of stalk to capsule there is a "jog" or S-shaped crook in the bundle (fig. 47). This curvature is in the sagittal plane and is somewhat less pronounced than in *Marsilea*. This appears to be due to the attachment of the stalk being nearer the middle of the dorsal side of the capsule than is the case in *Marsilea*, in which genus the stalk is attached toward the proximal end of the capsule.

The place of attachment of the stalk accounts also for the difference in plan of the vascular system of the capsule which *Regnellidium* presents. Figure 26 attempts to generalize the observations which have been made on this point by means of serial sections, checked up by a study of halves of capsule walls which were rendered transparent by the method devised by Stebbins (1938). The prominent feature is the series of rib-like "girdling bundles" which practically encircle the capsule. The course of these is intimately connected with the plan of the soral cavities, as illustrated in figure 25.

From its entrance into the capsule the peduncular bundle is continued forward in the dorsal region as a median bundle, at length forking to give rise to the last (generally the sixth) pair of girdling bundles. On each side of the median bundle the following branches are given off: (1) running outward from the median bundle for a short distance, then *backward*, finally forming the first girdling bundle, but meanwhile giving off the branches which are girdling bundles number 2 and 3; (2) girdling bundle number 4, (3) girdling bundle number 5. A short distance away from its origin each girdling bundle sends off a strong branch to a sorus, then forks into two almost parallel strands which continue around to the ventral side of the capsule where they anastomose with other strands of similar origin, from the same and also from the opposite side of the capsule. Hence it comes that there are about 12 bundles on each side of the capsule in the ventral region.

DEVELOPMENT OF THE SPOROCARP.—After the apical cell of a fertile leaf has cut off four or five pairs of segments, a sporocarp initial makes its appearance as a projection from one of the segments on one side of the young leaf (fig. 54). The initial arises probably from a marginal cell, although this point was not determined with certainty. In form and in the direction of its divisions it closely resembles the apical cell of the leaf, cutting off alternately right and left some 25 semicircular segments which in the region of the capsule are cut by six or more sector walls, leaving a marginal cell between the last two walls. So far, the development of the sporocarp thus resembles that of the leaf except in the possibly greater number of sector walls occurring in the former. Certain marginal cells distributed along the length of the young capsule next elongate radially and divide tangentially to give rise to the radial rows of microsporangia and megasporangia of the six (five to seven) sori in each half of the sporocarp. Meanwhile the sectors formed on the median and on the lateral sides of these marginal cells grow vigorously in the ventral direction (fig. 24, 52) and thus bury the sporangium initials deep within the capsule in soral canals, each of which for a time opens to the ventral surface of the capsule by a distinct pore (fig. 52). These pores persist until the capsule is over a millimetre in diameter, but eventually the edges of the openings come so close together that no trace of the pores remains. During the early stages the sporocarp has the form of a short, blunt-pointed cylinder; as the capsule makes its appearance it is more or less club-shaped, and the stalk shows the beginning of the bend in the basal (adaxial) direction (fig. 48); as the sori elongate, the capsule becomes more globular (fig. 47), and the stalk bends further, swinging to a total extent of at least 120° ; finally the capsule attains the form of a prolate spheroid which characterizes its adult condition, and the stalk unbends until the angle between stalk and soral axis is somewhere between 60° and 90° . The soral canals enlarge in all dimensions as the capsule matures, and the partitions between them become exceedingly thin. Somewhat loosely connected with these partitions are the elongated sori, consisting of a row of megasporangia lined along each edge by a row of microsporangia, all inserted upon the receptacle. The soral canals are present to the number of six (five to seven) on each side of a median partition; the capsule is accordingly seen to be distinctly bilateral. In a capsule 1 mm. in diameter the vascular bundles are plainly demarked. Their course has already been traced, but further reference should be made here to the strands that supply the sori. In the young capsule it can clearly be seen that soon after leaving the dorsal region, each of the girdling bundles sends off a "placental" branch into a receptacle rather than into a partition between soral canals. So much is this the case that in some sections the receptacle with its attached sporangia appears like a bunch of grapes hanging from the upper end of the soral canal, but, with the possible

exception of the sori at the proximal and distal ends of the capsule, the sori even in the young capsule are really attached to the partitions. Since the placental bundle enters at one end of the receptacle, there is here no forking of this bundle such as is figured by Johnson (1898, fig. 33) for *Marsilea*, in which case the placental branch arises from the median region of the girdling bundle.

In some capsules the median bundle shows a tendency to split beyond the point of origin of girdling bundle No. 4. Moreover, girdling bundle No. 3 may properly be considered to originate by dichotomy of the bundle called "branch (1)." Thus the plan of venation on each side of the median line may be interpreted as a series of dichotomies, strongly suggesting the venation of a pinna. A comparison of this plan with figure 135 of Eames (1936) shows that in *Marsilea* there are no backwardly directed bundles, all the sori being supplied from the forward extension of the peduncular bundle, which, moreover, is observed to fork instead of remaining a single median strand as we have seen to be the case in *Regnellidium*.

DISCUSSION.—The internal structure of the vegetative organs of *Marsilea* has long been known, due to the classic work of Russow (1872). Our examination of *Regnellidium* has shown a close correspondence, especially in the case of the stem. With respect to the petiole, both genera show the two large vessels, but in *Regnellidium* the transverse section of the xylem has the form of a wide open V, while in *Marsilea* the two arms of the V are separate and slightly curved away from each other (v. Russow, figure 26). This is a difference shown not only by various genera of ferns closely related to each other but even in different regions of the rachis in the same leaf. Attention may be called to the less differentiated condition of the xylem in the basal region of fertile leaves, shown in the absence of the two large vessels. The various organs show a combination of aquatic features, such as the array of intercellular canals, with terrestrial structures best illustrated by the well lignified tracheae, easily accounted for when one recalls the amphibious habit of the plant. The simple cart-wheel plan of the stem as seen in transverse section is probably connected with the small diameter—a wide stem such as is seen in many monocotyledons could not be constructed on so simple a plan.

In histogenesis, moreover, *Regnellidium* is found to be exceedingly similar to *Marsilea*, as made known by the earlier work of Johnson (1898). This is especially clear in the case of the petiole, where the successive divisions of the segments on each side take place with remarkable uniformity.

Concerning the precise relative position of the two pinnae on the petiole, it is evident that they cannot well be strictly opposite, since the segments from which they arise are alternate. The pinnae on the petiole evidently cannot be proven to be exactly opposite unless it can be shown that one or both pinnae involve fractions of segments. This has not

yet been demonstrated in *Regnellidium* though it is shown to be true in *Marsilea* (Johnson, 1898, p. 126). It is thus difficult to accept the conclusion of certain students of the Marsileaceae with regard to the four pinnae of the leaf of *Marsilea* as the result of three successive dichotomies (Bower, 1926, p. 178; Eames, 1936, p. 200). There seems to the writers no evidence from the development of the lamina that the two pinnae present in the leaf of *Regnellidium* correspond to the upper of the two pairs of pinnae of *Marsilea* as suggested by Eames (1936, p. 201). On the contrary, we find that the leaf of *Regnellidium* as in the case of *Marsilea* is still growing as a monopodium by an apical cell after the pinnae have been initiated.

We find no evidence against the usual view that with respect to leaves there is a reduction series: *Marsilea*, *Regnellidium*, *Pilularia*. Despite the lack of distinct evidence in its favor, it is tempting to entertain the idea that *M. polycarpa* represents a primitive member of the family.

Undoubtedly the chief subject of interest is the sporocarp. The evidence has become convincing that throughout the family the symmetry is bilateral, notwithstanding the superficial appearance of radial plan in *Pilularia*. The spheroidal capsule of *Regnellidium* stands intermediate between the elongated organ of *Marsilea* and the spherical one in *Pilularia*. This bilaterality is basic, since the sporocarp is from the outset a two-sided organ formed by the activity of an apical cell with two cutting faces. Moreover, the vascular bundle of the stalk is bilateral, while the architecture of the capsule is more so, with its soral canals lying right and left of the median partition. These canals, most numerous in *Marsilea* (about eight pairs in *M. quadrifolia*) are reduced to six (or five) pairs in *Regnellidium* and to two pairs or a single pair in different species of *Pilularia*.

The extent of curvature of the stalk in different members of the family deserves attention. This is best expressed in terms of the angle between the main portion of the stalk and the soral axis. It has already been shown that in *Regnellidium* the stalk of the growing sporocarp bends in the same direction as the sterile part of the leaf—that is, in what is commonly called the circinate manner. Later the curvature of the leaf tip disappears and that of the sporocarp lessens. The eventual angle between stalk and soral axis is about 30° in *Marsilea quadrifolia*, 90° in *M. salicifolia*, 60–90° in *Regnellidium*, and 160° in *Pilularia globulifera*.

As to the region of attachment of stalk to capsule, *Regnellidium* more nearly resembles *Pilularia*, since in both these genera the junction is on the dorsal surface of the capsule a short distance proximal to the mid-dorsal point, while in the various species of *Marsilea* the attachment is at some point on the proximal end of the capsule. In spite of this superficial resemblance of *Regnellidium* and *Pilularia*, the vascular plan in the former genus is more nearly like that of *Marsilea*. In both these genera the

prominent feature of the vascular framework is the set of rib-like girdling bundles, corresponding in number with the soral canals. It has been shown that the location of the point of entrance of the stalk exerts a determining influence on the plan of the vascular skeleton of the capsule. As to the distribution of vascular elements to the sori, it has been shown that in *Regnellidium* a vascular strand from one of the "ribs" enters the soral receptacle at its dorsal end and runs the length of the sorus, while it was already known (Russow, 1872) that in *Marsilea* the strand enters the sorus near the middle region and branches fore and aft, so to speak. This difference has been seen to be associated with the fact that in the latter genus the sori are attached to the lateral wall of the capsule, while in *Regnellidium* they are attached to the partitions which lie between the soral canals. Probably we see here an example of what has been called the "phyletic slide." Evidence as to which of the two places of attachment is primitive seems to be lacking.

Other features of the capsule which occur in all three genera are (1) the S-shaped bend in the vascular bundle of the stalk just as it enters the capsule; this bend is most pronounced in *Marsilea*. (2) The "overlap" of hypodermis just distal to the point of insertion of the stalk—concerning the significance of this curious feature all authors appear to observe a discreet silence. (3) The differentiation of the hypodermis as one or two "prismatic layers." These are most strongly thickened in *Marsilea*, but the curious periclinal line in the outer layer is evident in all genera. It is a remarkable fact, to which Russow (1872) has called attention, that prismatic layers occur also in the seed coat of certain Leguminosae. The great development of these layers in the dorsal part of the capsule in *Marsilea* may be connected with the more regular dehiscence in this genus compared with the irregular breaking up of the capsule wall in the other genera. Attention may also be drawn to the lack of a gelatinous ring in the opening capsule of *Regnellidium* and *Pilularia*. Finally, the two tubercles visible on the capsule of *Marsilea* are quite undeveloped in *Regnellidium*. Thus in several respects the capsule of *Regnellidium* is relatively undifferentiated.

In many respects it is seen that the genus *Regnellidium* occupies a place intermediate between *Marsilea* and *Pilularia*. This is the first impression one receives when looking at the leaves and sporocarp; the impression is confirmed by a study of internal structure.

The outstanding problem relative to the family is undoubtedly the morphological nature of the sporocarp. Several theories have been advanced, the best supported of which regards each valve of the capsule as the equivalent of a leaflet. Eames (1936) has clearly stated the evidence for this view and adduced a comparison with the leaflet of *Blechnum*.

The bearing of developmental studies is expressed by the following words, quoted from Johnson's report

in the 1937 Bulletin of Mount Desert Biological Laboratory: "There is here no more evidence from the development of leaf and capsule than is found in *Marsilea* for the view that the walls of the capsule are to be thought of as infolded pinnae. The enclosing wall of the capsule is in both cases developed in an entirely distinct manner, not at all closely like the process of development of the leaflets of the sterile leaf by the persistent formation of sectors on both edges of the very numerous marginal cells."

It will be well, however, to examine the evidence derived from anatomical studies. The lateral position of the sporocarp is shown first by the origin of its initial on one side of the median line of the young petiole, second by the observation that its vascular strand breaks off from one margin of the petiolar bundle, just as does the pinna trace in many of the more advanced families of ferns. The danger in attaching too much significance to this feature is shown by the fact that in *Marsilea* spp. a second sporocarp may branch off from the stalk of the first one. Probably we see here merely an expression of the fundamental tendency to dichotomy seen throughout the fern group, resulting frequently in sympodial growth. The vascular skeleton of the capsule in *Marsilea* presents every appearance of a sympodium, while *Regnellidium* shows a clearer series of nearly equal dichotomies (see fig. 26). As we have pointed out, the venation on each side of the capsule in *Regnellidium* definitely suggests that of a pinna, for the position of insertion of the stalk on the capsule enables the essential dichotomy to express itself in a less specialized way than is seen in *Marsilea*. The vascular anatomy of the capsule therefore supports the view that two leaflets enter into the construction of the capsule, as they do also in the sterile leaf. The whole plant is to be regarded as an expression of a series of dichotomies, frequently taking the form of a sympodium. The relation of these considerations to the current theory of origin of the fern body by "overtopping" is obvious.

SUMMARY

Regnellidium, monotypic genus of Marsileaceae, has been rediscovered in southern Brazil, and material suitable for detailed study has been secured.

The internal structure of stem, leaf, and root is found to correspond closely with that of *Marsilea*. The morphology of the vascular tissues is very similar to that of many other leptosporangiate ferns.

Growth of stem, leaf, and sporocarp is initiated by division of apical cells with two cutting faces, while the tissues of the root arise from a cell with four cutting faces.

The bifacial initial of a leaf cuts off 15 or more segments to the right and a similar number to the

left. Each member of the 10+ pairs of segments that go to form the petiole divides by approximately radial walls to "sectors" which, with the "marginal cell" remaining between the last two sector walls, are then divided by periclines that separate epidermis, cortex, and vascular bundle.

On each side of the petiolar bundle is a large vessel which is formed from a series of cells belonging to sector II of the various segments. Each cell of this series, contrary to its neighbors, does not divide either transversely or longitudinally, although its nucleus may divide as many as four times.

In the 3 or 4 segments of each side which go to form the pinnae, the marginal cells retain the power of splitting off sectors which then rapidly extend radially and divide in two planes so as to produce the fan-shaped pinnae.

In its origin and in the insertion of its vascular bundle the sporocarp is seen to be a lateral organ. In spite of the approximately spherical form of the capsule, its symmetry is strictly bilateral.

Development of the sporocarp follows for a time the same course as the petiole. Then on each side of the tip region certain marginal cells cut off soral initials, while the adjoining sectors elongate radially in such way as to leave each soral initial imbedded. Each sorus is surrounded by a soral canal which for some time opens to the ventral surface by a pore.

The vascular system of the capsule is similar to that of *Marsilea*, but because the attachment of the stalk is on the dorsal side of the capsule, the venation in *Regnellidium* is more obviously a series of dichotomies resembling the plan seen in the pinna.

Attachment of the soral receptacle to the partitions lying between the soral canals is found to characterize the capsule at all stages. The vascular supply of each receptacle enters at the end, not the side. In both these respects *Regnellidium* differs from *Marsilea*.

The wall of the mature capsule shows the same peculiar thickening of the hypodermis as *Marsilea*, but is not so highly specialized. The peculiar "overlap" which is found in the other members of the family occurs here also.

In its general features and in details of structure *Regnellidium* is intermediate between *Marsilea* and *Pitularia*. No trace of more than two leaflets has been found, and the sporocarp is uniformly single. The vascular structure of the capsule strongly suggests that it represents a pair of pinnae.

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THE COMBINED EFFECTS OF THE DOMINANT AND THE RECESSIVE LETHALS FOR ASCUS ABORTION IN NEUROSPORA¹

B. O. Dodge and Bernice Seaver

THE ACTIVITIES within the ascus of *Neurospora tetrasperma* which culminate in the formation and discharge of the ascospores comprise two developmental phases. The first begins with the fusion of two nuclei of opposite sex when the ascus is very young. The ascus cell then increases considerably in size, after which the fusion nucleus undergoes the reduction divisions. The four resulting nuclei become characteristically oriented, and a third division, which is equational, completes the first phase.

The second phase begins with the development of the astral ray system. The ascospores are delimited, and soon after, all the nuclei, two in each of the four spores, simultaneously undergo a fourth division. The spore wall becomes thickened, dark olivaceous brown, and striated. When fully mature the spores are discharged rather violently through the pore at the apex of the ascus. If development ceases at any time between the nuclear fusion and the end of the third division, the ascus may be said to abort. If spores are delimited but fail to mature, then it is the spores that abort and not the ascus.

DELINQUENT ASCUS ABORTION.—There are two types of ascus abortion, both genetic and heritable, and both have been previously described (Dodge, 1934, 1935). The first may be referred to as delinquent or cytolytic abortion (fig. 1, B). The full quota of asci arise in the ascocarp and become well elongated. Then, without cutting out any spores whatever, they all slowly disintegrate and disappear. This occurs when both parents in the mating carry a certain lethal factor so that all the asci in every ascocarp will be homozygous for this lethal. When

one of the parent races is normal, or of the wild type, and the other carries the lethal so that all asci are heterozygous, spores are formed normally. We have here a perfect example of simple mendelian dominance.

INDURATED ASCUS ABORTION.—The second type of abortion has been referred to as indurated abortion. Asci that abort and persist become chitinated or indurated, dark colored, and striated like the ascospores themselves (fig. 1, C). This occurs when only one of the parent races carries a certain other lethal factor. Since all the asci resulting from the mating are heterozygous for this lethal, it must be dominant in those asci that abort. There are always in every ascocarp some asci that mature spores, without which genetic studies could not be carried on beyond the first generation. We shall refer, however, to this lethal for convenience as dominant, although, so far as the whole output of asci is concerned, it is only more or less dominant.

THE PROBLEM.—It was desirable for several reasons (to be noted later) that we should develop races which, when mated, would mature ascocarps in which all asci would abort and all the asci that persisted would become indurated. It is clear that if both parents carried the recessive lethal and one of them also carried the dominant lethal, we might obtain the desired results provided both lethals acted independently and there was no linkage or other interference.

PRINCIPLES INVOLVED.—We shall digress to explain some of the principles involved as well as the difficulties encountered in making the necessary crosses, because the information finally obtained was perhaps as important from another standpoint as the results originally desired.

The two types of abortion have been previously studied cytologically and reported on in papers cited above. In an ascus homozygous for the recessive

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lethal the eight nuclei following from the third division come to the central region and gradually degenerate. There is no question of the nuclear behavior in such asci. In asci heterozygous for the dominant lethal which causes the indurated type of abortion, nuclear behavior has not been sufficiently studied. There is evidence, however, that the eight nuclei, instead of degenerating, are stimulated to go into the fourth division precociously, with the result that one often sees what appear to be at least sixteen nuclei in an aborting ascus. With the two lethals acting with directly opposite effects, as it were, one could not predict the outcome without actually making the crosses necessary to obtain asci homozygous for the recessive lethal and heterozygous for the dominant one.

The recessive lethal can be carried along very easily in unisexual haploid races so that matings with it can be made at will. Even wide interspecific crosses involving this lethal have been made (Dodge, 1936).

Carrying the dominant lethal in unisexual haploid races is a very different thing, as one would suspect. Small spores having only the lethal nucleus often germinate, but their dwarf mycelia soon die. In case of the larger spores characteristic of 4-spored asci, each spore is binucleate and bisexual at its origin. When one of the nuclei in such a spore carries the dominant lethal and the other does not, the spore will germinate fairly normally. The nucleus carrying the lethal will continue to divide and take part in extended mycelial growth since it is always accompanied by its normal partner. When this work was begun, we had no unisexual race which carried the lethal by itself. The original Uber and Goddard race (Dodge, 1934) has usually reacted as unisexual, although actually bisexual, but it had become attenuated with long storage in the refrigerator. It was later rejuvenated and used successfully in making further crosses. It was necessary, however, in beginning this work, to use as one of the parents a bisexual

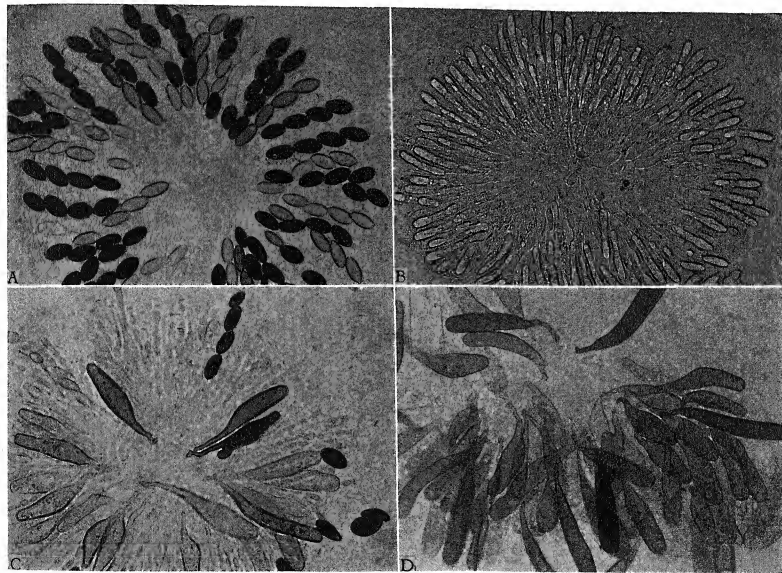


Fig. 1.—A. Asci originally heterozygous, *AaiDd* for the recessive lethal, *d*. Normal asci, *AaiDD*, would present exactly the same appearance.—B. Asci originally homozygous, *Aaiidd*, for the recessive, *d*. Deliquescent ascus abortion. No spores are delimited. Asci such as shown here finally disintegrate and disappear.—C. Asci originally heterozygous, *AaiDd*, for all three pairs of factors. Some asci delimit spores. The indurated ascus abortion is due to the lethal *I* which is strictly dominant in aborting asci. Referring to the whole quota of asci, *I* is only more or less or incompletely dominant because some asci do delimit spores.—D. Asci originally heterozygous, *Aaiidd*, for *I* and homozygous for *d*. Total abortion, no spores. Asci become indurated. The ascus is a diploid structure as soon as its two nuclei of opposite sex fuse. This occurs when the ascus is very young. In this paper when we refer to an ascus as homozygous, we are considering its condition before reduction occurs.

or hermaphroditic self fertile race, such as could be obtained by germinating the large bisexual spores from ascocarps which also developed indurated asci.

SYMBOLS.—In this work the letters *A* and *a* represent the sex factors; *I* and *i* are the factors concerned with the indurated type of ascus abortion, *I* being lethal and more or less dominant; *D* and *d* are the factors involved in the *deliquescent* type of abortion,² *d* being recessive and lethal. We use these symbols for convenience merely to designate the factor complexes, whatever they may be, without implying that they represent single genes. The genotypes of the two kinds of nuclei in a bisexual race which carries the factor *I* in one of its nuclei will be either *AID* for one nucleus and *aiD* for the other, or *AiD* and *aID*, respectively. Our tester races 97C4 and 97C8, referred to in this paper simply as C4 and C8, are *Aid* and *aId*, respectively.

The first step, then, was to replace the factor *D* with *d* in that nucleus carrying the dominant lethal *I* so that the genotype of our two kinds of nuclei in a bisexual spore (and its mycelium) of the next generation would be either *Aid* and *aId* or *AiD* and *aId*. That is, we had to induce matings with one of our unisexual races either *Aid* or *aId* as one parent, in a way that would give asci *AaiDd*, hoping that the lethals would segregate regularly, giving, among others, the new combinations desired. We know in advance the genotypes *Aid* and *aId* of our unisexual parent races, but we do not know which one of the two kinds of nuclei in our bisexual race carries the dominant lethal *I*.

² The letters *L* and *l* were employed in earlier papers to represent these factors.

NUCLEAR MIGRATIONS AND HYPHAL INVASIONS.—The method has been described by which a normal bisexual race in which every cell is presumed to contain compatible nuclei of opposite sex can be crossed with a normal unisexual race (Dodge, 1931). Cultures in which bisexual races carrying the factor *I* were grown opposite normal races $S_1 = aiD$ and $S_2 = AiD$ were mentioned in an earlier paper (Dodge, 1935, p. 118). The question was whether or not such crosses could be made when the bisexual parental race which carries the dominant lethal, *I*, in one kind of its nuclei is grown opposite a unisexual race which carries the recessive lethal, *d*, in all its nuclei. It is known that when two unisexual races of opposite sex are grown from opposite sides in plate cultures, the nuclei of one sex migrate through the openings at the points of anastomosis and then pass down along the hyphae through the openings in the cross walls so that parts of the mycelium of at least one of the original unisexual races in the mating become heterocaryotic and bisexual, a condition finally essential for ascocarp development. There is certainly no wholesale intermingling of hyphae of both kinds over plate cultures of these unisexual paired races as we prepare them, but it would be difficult to prove that there is not some invasion of one side by hyphae from the other side of the culture.

When a bisexual and a unisexual race were crossed, as stated in the paper referred to (Dodge, 1931), it was not known whether crossing came about as a result of nuclear migration or because of hyphal intermixing. It will be shown presently that the normal nuclei, *AiD*, in composite mycelia, (*AiD*, *aId*), freely abandon the *aId* nuclei and migrate over to mate

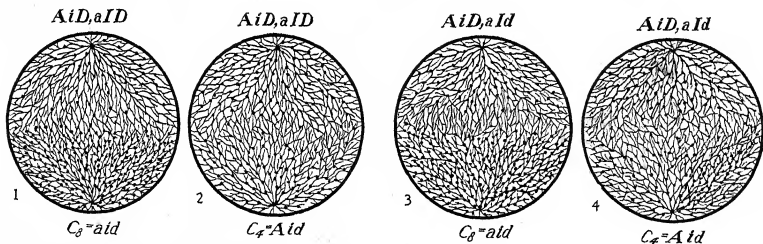


Fig. 2. Diagrams indicating the method of procedure in crossing a bisexual race (in which the nuclei of one sex carry the dominant lethal *I*) with the unisexual race, all the nuclei of which carry the recessive lethal *d*; the dots represent ascocarps. At the top of each diagram ascocarps resulting from selfing would contain asci, some of which would abort and become indurated, while other asci in the same fruit body would develop spores. Matings can occur only where *A* and *a* nuclei enter a primordium. No. 1 shows distribution pattern where practically all the ascocarps would contain 4-spored asci. This could only mean that *AiD* nuclei migrated over from above, leaving their *aID* partners to mate *AiD* \times *aId* on the *C*₈ side. If nuclei of one sex in the bisexual race are *Aid*, those of the opposite sex must be *aID*. In No. 2 all ascocarps would look alike, having indurated asci and some spores, and only by testing the spores from individual ascocarps on the *C*₄ side could one tell whether they resulted from a cross, *aID* \times *aId*, or from selfing, *Aid* \times *aID*, where both kinds of nuclei in some way, either by mycelial invasion or double nuclear migration, got over into the *C*₄ side. Such a test is represented in Nos. 3 and 4. In No. 3 many ascocarps on the *C*₈ side would contain 4-spored asci, no ascus abortion. Again the *AiD* nuclei must have migrated across from above. The other nuclei of the bisexual race must have been *aId*. If so, as shown in No. 4, nuclei, *aId*, would migrate across to mate with nuclei *Aid* to give us the desired cross where all asci would be heterozygous for the dominant lethal and homozygous for the recessive lethal.

with the *aid* nuclei in the unisexual race. Likewise *aid* nuclei leave cells carrying *AID* nuclei to mate with *Aid* nuclei over on the other side. The behavior of nuclei of various sorts in their relationship to nuclei carrying *I* is very striking from the physiological as well as from the genetic standpoint. Nuclei of the genotypes, either *AID* or *aID*, are not always as free to migrate unless they are accompanied by their original associates, either in actual migration or coincident with hyphal invasions, and thus be able to take part in ascocarp development through selfing. Such behavior was discounted in advance, and our only hope was that some of the *AID* nuclei, for example, would desert their normal *aid* associates at the first opportunity and go over to the opposite side and mate with *aid* nuclei. We may report here that this certainly does happen under some conditions, so that the desired results were finally obtained, although it would have been less difficult if we had started with the Uber and Goddard race G5.3 which reverts as unisexual, *AID*. Cultures obtained from matings of this race will be described later.

CULTURE EXPERIMENTS.—Testing spores from dried stock cultures.—A tube culture which had been dried in the laboratory since June 8, 1934, furnished the ascospores which carried the dominant lethal *I*. This was tube number 4.11.18.1, which for convenience will be referred to here merely as 481. The ascospores were of the fourth filial generation from the original mating G5.3 × 81 (Dodge, 1934). A number of these spores were sowed on corn meal agar plates, heated the usual way at about 58° for one hour, and set aside. It usually requires about four hours for germination of normal ascospores to begin. It was found that the spores from this culture required a much longer period, about twenty-four hours in the incubator. The germ tubes were still short enough to transfer without danger of including germ tubes from neighboring spores. Of the fifty germinating spores transferred, twenty-eight developed rather normal and vigorously growing mycelia. Twenty-two died soon after being transferred without making any appreciable additional growth. The presence of the dominant lethal nucleus in an ascospore long exposed to drying may have some detrimental effect on the normal nucleus also in that spore. Even though the lethal nucleus failed to divide, the normal nucleus otherwise should have furnished the energy for the development of a good mycelium, especially after the spore had germinated. Seventeen of the twenty-eight that lived proved to be bisexual, and every ascocarp examined in each culture had many indurated aborted asci and, of course, a few spores. This certainly proves again that this type of abortion is heritable even after the spores carrying the lethal have been dried for four years.

Testing for sex.—In order to find out for each race which one of the two kinds of nuclei carried the factor *I*, each of fifteen of these races was grown in

two plate cultures, inoculations being made at one side. The opposite side of one of the plates was inoculated with our C4 tester, and C8 was placed in a similar position in the other plate. Ordinarily, in simply testing for sex, we would have used our normal testers S6 and S1 instead of C4 and C8 which carry the recessive lethal *d*. The latter were used because, while testing for sex, we could just as well try to make the first necessary crosses involving both lethals, *I* and *d*. The diagram (fig. 2) indicates in a general way how testing is done, always in pairs. For example, in testing race 481.3 the pairings would be 481.3 + C4 and 481.3 + C8, C4 and C8 being *Aid* and *aid*, respectively, as noted previously. In this test a great many ascocarps matured on the C8 side of one plate within ten days, and these fruit bodies all contained 4-spored asci with no indurated abortion. Regardless of what appeared on the C4 side in the other test plate, this proved that *AID* nuclei had migrated out from the bisexual mycelium 481.3 side to mate with our tester C8 in the cross *AID* × *aid*. Such matings give asci *AaiDd*, heterozygous for the recessive lethal, so that spores will be cut out normally without any ascus abortion. Furthermore, since one of the two kinds of nuclei in 481.3 had the genotype *Aid*, the other kind must have all been *aid* because ascocarps bearing indurated asci were formed when 481.3 was grown by itself. Race 481.3 is (*Aid*, *aID*). The genotypes of eight of the fifteen spores tested in this way were (*AID*, *aid*) and seven spores were (*Aid*, *aID*).

When a bisexual race proved by test to be (*AID*, *aID*) was grown opposite our *Aid* tester C4, all perithecia examined, with few exceptions, had 4-spored asci, with no indurated ascus abortion. Nuclei *aID* had left *AID* nuclei to enter the cross *aID* × *Aid*. When this same race (*AID*, *aID*) was grown against our *aid* tester C8, all perithecia on the C8 side had some indurated aborted asci, but other asci in the same fruit body developed spores. If what may be called normal nuclei, *Aid* and *aID*, freely leave their natural mates, *aID* and *AID*, respectively, in bisexual mycelia and migrate over to mate with nuclei of the opposite sex *aid* and *Aid*, in crosses either *AID* × *aid* or *aID* × *Aid*, one might be pardoned for hoping that the *aID* and *AID* nuclei would also migrate freely.

Testing *f*₁ progeny for the presence of the recessive lethal, *d*.—When race 481.3 which is (*AID*, *aID*), as noted above, was grown opposite *Aid*, tester C4, the fruit bodies on the C4 side (from which fifty spores tested later happened to have come) were proved to have been formed as the result of selfing either through mycelial invasions of the *Aid* side by the bisexual self-fertile race (*Aid*, *aID*) or because of a migration of both kinds of its nuclei through hyphae of the tester. This was proved by the fact that the recessive factor *d* did not appear in races from any of the *f*₁ progeny spores tested. However, when race 481.13, which is also (*Aid*, *aID*), was grown opposite the same *Aid*, tester C4, the factor *d* was found in all the bisexual *f*₁ progeny spores tested. At least some

of the fruit bodies on the C4 side were the result of a cross $aID \times Aid$, which was the first step necessary in the solution of our problem (see fig. 2, 2). No doubt this occurred on the C4 side in the plate where 4813 was tested, though we happened to pick spores from the wrong ascocarps on the C4 side. Figure 3 shows a test plate where two kinds of ascocarps were found in the tester C4 side as explained in the legend. It is clear that it is only by breeding the f_1 progeny with the Aid and aId testers in backcrosses that the presence or absence of the factor d in a bisexual race will be made manifest.

Ascocarps from a cross $AiD \times aId$ look exactly like those from the selfing of (AiD , aID), because both kinds develop some indurated aborted asci and some other asci that produce spores. It was later discovered, however, that one can readily distinguish by their external appearance the difference between ascocarps from a selfing in a race (AiD , aId) and those produced by a cross $Aid \times aId$ where the aId nucleus from the bisexual race leaves its normal AiD associate to mate with the Aid tester. Ascocarps



Fig. 3. Plate culture showing the ascocarp distribution pattern where a self-fertile bisexual race (AiD , aID), above, is grown opposite the tester C4, Aid , below. All ascocarps above were presumed to have been the result of selfing, $AiD \times aID$. They contained indurated asci and some spores. Ascocarps below were proved to be of two kinds which could not be distinguished either by their external appearance or by microscopic examination. By testing mycelia derived from their spores in backcrosses with testers Aid and aId , it was proved that certain ascocarps were the result of selfing, the bisexual mycelium from above having invaded the lower side. Other ascocarps on this side were proved to have arisen from a cross $aID \times Aid$, indicating a straight nuclear migration of aID across to mate with the tester Aid . Progeny bisexual spores carried the factor d in one of their two kinds of nuclei.

from selfing, in this case $AiD \times aID$, always develop some spores as well as indurated aborted asci; the necks of those ascocarps are usually more conspicuous, and when mature, numbers of spores can be seen around the ostiolar region, especially when the fruit bodies are developed beneath the surface of the medium. When the fruit body is the result of a cross, $Aid \times aId$, no spores are ever formed; all asci abort, and those that persist become indurated (fig. 1, D). Both lethals seem to act independently.

Some 53 bisexual spores in this series were analyzed in the manner described above, and each spore was proved to carry d in one of its two nuclei. Six of the races from these spores were so self-infertile that the nuclei carrying I readily left their original associates to cross with our testers to give rather pure cultures in which both parents carried the recessive, d , and one of them the dominant, I . For example, a tester mycelium which was originally unisexual, Aid , becomes bisexual, (Aid , aId), through migration of aId nuclei from the opposite side of the plate. Transfers from an area that has thus become diploidized (not diploidized) provides the race sought.

Crosses involving the Uber and Goddard race G5.3 as one parent.—Race G5.3 derived from an irradiated ascospore reacts as unisexual, AID , although it was originally, and still is, very weakly bisexual (AID , aId). When grown by itself, however, it now produces no ascocarps.

Plates in which G5.3 was grown opposite our C8, aId , for several weeks finally matured a few ascocarps which produced some spores, while other asci were of the indurated aborted type. This showed that the race had crossed with our race bearing the recessive lethal, but perhaps not as readily as it would have crossed with our normal S_1 tester, aId . Since all asci must have been heterozygous, $AaiDiD$, for all three pairs of factors, eight different kinds of nuclei should have been found in f_1 progeny spores. The genotypes of the nuclei would be AID , AiD , AiD , Aid , aID , aId , aID , aId . Since two nuclei normally go to each spore, there could have been 36 different kinds of spores. Usually only nuclei of opposite sex go to the same spore. This would give us 16 kinds of spores. But spores in which both nuclei carry the lethal I would die soon after germination, and it is only rarely, because of the peculiar mechanics operating in these 4-spored asci, that the two nuclei in a spore would both carry either D or d , (Seaver, 1937; Campbell, 1937).

After testing a number of the f_1 progeny from the mating $G5.3 \times C8$, which is the same as $AID \times aId$, we found in one series the following types of spores represented; the number of each kind follows the symbol: AID , aId (8); AiD , aId (18); Aid , aId (10). aId , aID (8); AiD , aId (1). Several unisexual spores were also analyzed. The following types of unisexual spores were found in the same series: AiD (3); Aid (3); aID (9); aId (4). Twelve spores germinated but died soon after. These no doubt carried only I nuclei. Adding to these the numbers

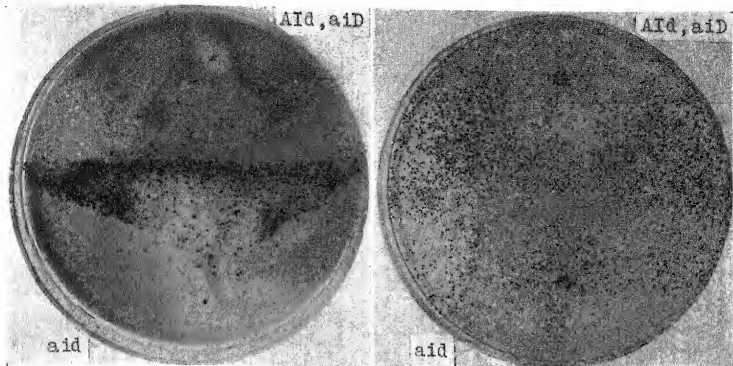


Fig. 4. Individual tests, not pairs. Left, distribution pattern where a self-infertile bisexual race (*AId, aId*) was grown opposite tester *aid*. All ascocarps contained only indurated asci, no spores. Right, a self-fertile bisexual race (*AId, aId*), above, produced its own ascocarps and also invaded the lower side to produce some of the same kind of ascocarps. The nuclei *AId*, however, frequently left their normal partners *aId* and migrated over to mate with tester *aid*. One can readily distinguish the two kinds of ascocarps on the lower side by their superficial appearance, as stated in the text.

obtained from the first series described, we have the following types of spores: *AID, aid* (17); *AId, aId* (38); *AId, aid* (28); *Aid, aId* (26); *AId, aid* (1); *Aid, aid* (2). Neither nucleus in each of the spores falling in the last two types carried the lethal *I*, and both lethals were excluded from the spores of the last type.³ The following include all types of the unisexual races analyzed: *AID* (20); *Aid* (7); *aId* (22); *aid* (19). Several races that carried *I* and at first were thought to be strictly unisexual were studied. These races together with G5.3 are the ones that should be used in obtaining further data on linkages because there is no danger of mistaking spores from selfing for spores from a cross.

Eight of the bisexual spores were somewhat self-infertile so that the nuclei carrying both lethals, *I* and *d*, migrated freely over to mate with nuclei on the opposite side of the plate which carried the *iD* factors. For example, race (G5.3 × C8) 2.11 remained sterile for a long time and tested as *AId*, a very desirable race, but like several others previously mentioned, finally proved to be very weakly bisexual (*AId, aId*). Where the bisexual parent in the plate tested was rather self-infertile, the perithecial distribution pattern was usually much like that shown in figure 4, left. This shows the pattern where (G5.3 × C8) 1.18, which proved to be (*AId, aId*) and self-infertile, was grown at the top, and tester C8, *aid*, was grown below. All fruit bodies in this plate both at the top and on the C8 area at the bottom were the result of a cross *AId* × *aid*. All asci were therefore *AaIdid*, homozygous for the recessive and heterozygous for the dominant lethal, *I*. All asci aborted

without any spore formation whatever, and all asci that persisted became indurated, as shown in our figure 1, *D*. Subcultures from this plate have continued to produce the same kind of ascocarps. At the right in figure 4 is shown the pattern where a self-fertile race [(G5.3 × C8) 2.8 × C4] 5, which also proved to be (*AId, aId*), was grown at the top and opposite C8, or *aid*. Some of the fruit bodies on the lower side contained only indurated aborted asci and no spores, while certain other ascocarps present produced some spores as well as indurated asci. The latter type must have resulted from hyphal invasion from the bisexual side above. The more self-fertile the bisexual race to be tested is, the less favorable it is for breeding work, because its mycelium tends to invade the other side of the plate more extensively. The presence of numbers of perithecia on the C8 side which could only have come from selfing is positive proof either for such hyphal invasions or for a double nuclear migration where the lethal nucleus carries its normal partner nucleus with it as it migrates down the hyphae of the unisexual race.

Testing for hyphal invasions.—Fourteen bisexual spores from various cultures which showed ascocarps with some spores where, theoretically at least, no spores should have been formed, were tested for the factor *D* in one of its two kinds of nuclei. In every case this *D* was found, so that those ascocarps must have resulted from selfing, otherwise the asci would all have been *AaIdid*. Furthermore, 23 unisexual spores were tested for *D*. Twelve had nuclei carrying *D* and eleven had nuclei which carried *d*. What at first appeared to be a suppression of *d* by *I* was easily explained on the theory of mycelial invasion or

³ One spore of the type *Aid, aid* was found later.

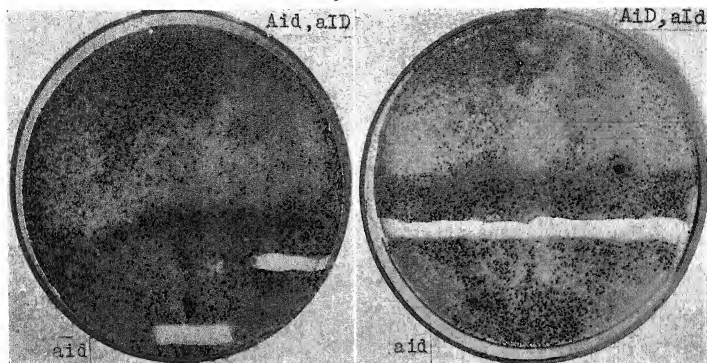


Fig. 5. Distribution patterns, individual tests, not pairs. Left, a self-fertile bisexual race, proved by the test to be (*Aid*, *aID*), was grown opposite tester *aid*. Most of the ascocarps below contained only deliquescent asci, showing that the *Aid* nuclei above deserted the *aID* nuclei and migrated down the hyphae of the tester to mate, *Aid* \times *aid*. About one per cent of the ascocarps taken out as indicated were suspected of being the result of a bisexual hyphal invasion from above because they contained indurated asci. The two kinds of ascocarps could be easily distinguished by their external appearance at maturity. The one per cent produced a few spores, the others did not. Right, practically all the ascocarps below contained 4-spored asci, with no abortion. Only one fruit body out of five hundred located on the agar strip removed contained indurated aborted asci and some spores. It would have been difficult to distinguish such a fruit body from the others without crushing them, because both kinds produced spores. Ascocarps above in both cases were the result of selfing as all contained indurated asci and some spores.

double nuclear migration. It would not be at all difficult to prove which of these two activities was responsible for the ascocarps due to selfing. One could readily isolate fragments of single hyphae from the area in which the two kinds of ascocarps had matured. If in the new cultures two kinds of fruit bodies were developed, one kind containing only indurated asci, then we could be sure that a double nuclear migration had occurred. If ascocarps with some spores and indurated asci were the only kind found, then this must have been due to hyphal invasions from the bisexual race being tested.

Apparent reversion of the factor I.—Having obtained bisexual races which produce only asci of the genotype *Aalidd* and which always abort and usually become indurated without ever forming any spores we are in a better position to study the question of a possible reversion of *I*, which has arisen several times in the past. When the first experiments with *I* were reported, it was thought that irradiation of the original ascospore G5.3 (Dodge, 1934) had resulted in a mutation due to a deficiency. The fact that uninucleate ascospores and conidia bearing *I* often germinate but die soon after would suggest this. If one watches a stock of cultures of a bisexual race such as (*AID*, *aID*) for several weeks, or until they are somewhat dried out, one will find some tubes where certain ascocarps have discharged masses of black spores. Such ascocarps when examined show no indurated ascus abortion. Furthermore, races de-

rived from their spores are normal and show in turn no ascus abortion, suggesting that the *I* factor has reverted to *i*, or has lost its potency to bring about induration. The great majority of ascocarps in the old cultures will contain indurated asci, and a few spores are always shot out of them, but never enough to make a visible spore print on the tube. A spore print is formed only by spores shot from apparently normal ascocarps in the culture. Their presence could be explained if it were proved that ascospores germinate in the same cultures that produce them. With even a limited mycelial growth there would be an opportunity for two normal nuclei of opposite sex to come together in an ascocarp primordium. The percentage of these old cultures showing normal ascocarps is usually too high to be explained on the basis of reversion.

Contamination of the old cultures is not to be considered as an explanation. We have observed hundreds of the old cultures where both parents in the matings carried the recessive lethal so that only deliquescent aborted asci were produced. None of these cultures has ever developed normal ascocarps with 4-spored asci. The production of 4-spored asci would be good proof of a contamination or of a reversion, in this case, *d* to *D*.

If a reversion of *I* now occurs in our new races which produce asci of the genotype *Aalidd*, it will be made manifest by the presence of ascocarps the asci of which will show the nonindurated deliquescent

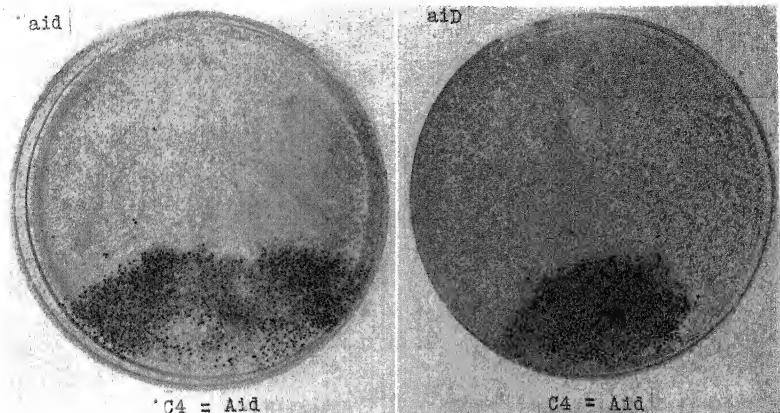


Fig. 6. Distribution pattern where two different unisexual races were tested for the presence or absence of *d*. Left, all ascocarps contained only deliquescent aborted asci, the cross having been *Aid* \times *aid*. Right, all asci in all perithecia contained 4-spored asci, the cross having been *AiD* \times *aid*. The genotype of the race is manifested by its reaction with the testers.

type of abortion. These asci will still be homozygous for the recessive lethal *Aa(i)idd* so that no spores could be delimited.

Nuclear behavior in indurated asci.—As stated earlier, cytological studies of indurated asci have not been carried out to any great extent, because there has not been found any way by which asci that would have aborted can be distinguished from asci that would have cut out spores. Our new races derived from such crosses as *Aid* \times *aid* and *Aid* \times *aid* will provide valuable material for cytological study because every ascus aborts and one can determine the effects on nuclear behavior of the two lethals working together in the same ascus.

Linkage relations.—The geneticist is most interested in possible linkages and chromosome maps, and he would want to know whether the factors *I* and *d* are sex-linked or are linked together in any way. Former studies (Dodge, 1936) gave no evidence for a linkage between *d* and the sex factors, *A* and *a*. Results of our present study likewise furnish no evidence for a linkage between *I* and the sex factors or between *I* and *d*. Discarding all results except those concerning which there could be no possible question as regards *ID* and *Id*, we obtained 50 old combinations to 58 new combinations. In case of *AI*, *aI* linkage possibilities our results show 84 old combinations to 80 new combinations. Relative to *Ai* and *ai* we obtained 126 old combinations to 125 new, which proves what we have always found to be true, that the sex factors segregate 1:1. Now that the technique for hybridizing bisexual races carrying *ID* or *Id* with unisexual races carrying *d* has been worked out, it

will not be difficult to obtain sufficient data to settle the question of linkages here. At present we believe that the lethals segregate independently both in the 1:1 ratio the same as the sex factors *A*, *a*.

Certain ascocarps in the test plates developed groups of asci which we suspected of being *Aaiidd*, while other asci in the same fruit body were *AaiDd*, due possibly to the inclusions of more than one pair of nuclei in the perithecial primordium. Furthermore, where two different kinds of ascocarps were found on the tester side of the plate where we expected only one kind, this was explained by assuming that there had been some interference with the factor *d* by *I* which upset the orderly processes of segregation (see Amer. Jour. Bot. 24: 731. 1937). There is certainly a very interesting relationship between nuclei of one sex carrying the factor *I* and nuclei of the opposite sex either with or without *d*, but lacking *I*, in their activities in sexual reproduction.

The history of the discovery of the recessive lethal, *d*, (Dodge, 1935, p. 117) would suggest that perhaps *d* may be linked with *I* in some way, because both lethals developed in race G5.3, which was obtained by germinating an ascospore that had been irradiated. The presence of an occasional indurated ascus among those of the deliquescent type in ascocarps first developed by race 9.7, as described in the paper just referred to, would suggest a possible linkage of some sort between *I* and *d*, although this may very well have no significance whatever as regards a linkage.

Can a race with all nuclei carrying I live?—Whether or not a race with only nuclei which carry the in-

completely lethal *I* can exist by itself in the vegetative condition for any length of time is still a question. During this work we obtained several races which we thought at first were unisexual and carried only *I* nuclei. Invariably, however, after several weeks the cultures gave rise to a few ascocarps with spores and

indurated aborted asci, proving that they were bisexual. Such races are most desirable for breeding because, within certain limits, they react very well as unisexual haploids. The bisexuality of these races was further proved when attempts were made to mate the one against the other. For example, when

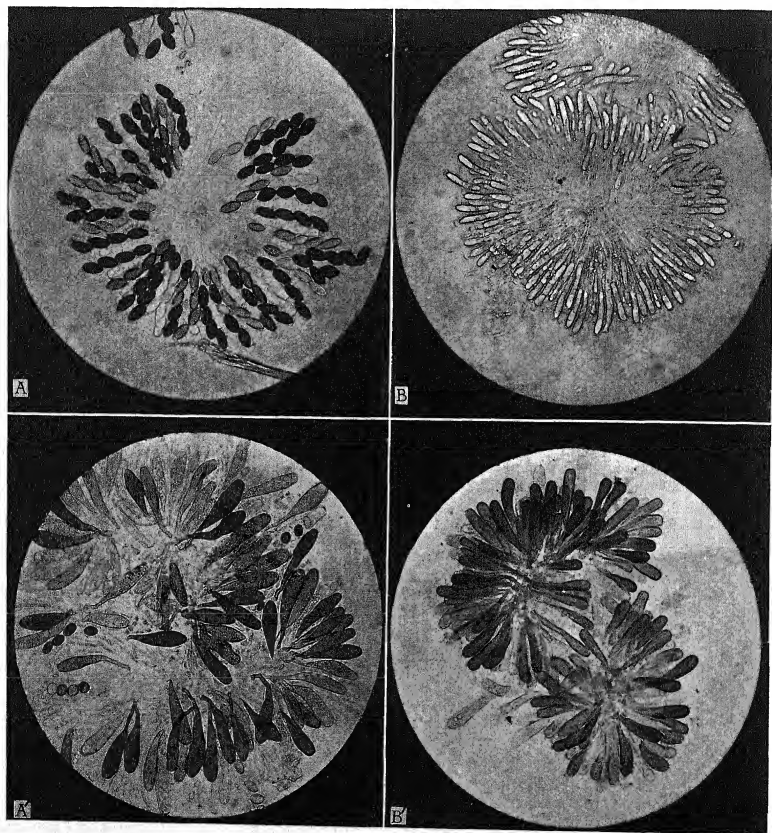


Fig 7.—A. Asci which were heterozygous *AaiiDd* for the recessive lethal, *d*, cut out spores normally.—B. Asci homozygous for *d*, all abort and finally disintegrate and disappear; no spore formation.—A'. Asci originally *AaiiDd*, the same as shown at A, yet most of them aborted and became indurated due to some unknown environmental condition. A few asci cut out spores. The non-heritable effect of the unfavorable environment was exactly the same as would have been induced had the incompletely dominant lethal *I* been present. Subcultures from these cultures produced asci, all of which cut out four spores as shown at A.—B'. Asci originally *Aaiidd*, but due to the same unfavorable environmental condition, all became indurated. They would have all become aborted but not indurated under the usual environmental culture conditions. Subcultures from this lot produced asci like those shown at B.

race ($G5.3 \times C8$) 2.11, which reacts as *aiD*, was grown in each of five plates opposite race ($481.13 \times C4$) 10, which reacts as *AiD*, two kinds of ascocarps were produced where only asci of the genotype, *Aaiidd*, if any at all, should have arisen if both races were unisexual. A few ascocarps in one plate contained only 4-spored asci and no indurated abortion, thus proving that the races were actually (*AiD*, *aiD*) and (*AiD*, *aiD*), respectively. Nuclei *aiD* of one race had mated with *AiD* of the other. Two or three ascocarps which contained only indurated asci were found, showing that it may be possible to obtain asci homozygous for both lethals, *AiD* mating with *aiD*. The evidence furnished by these preliminary trials, however, is too meager to warrant a final conclusion because there must come a time when, as the ascus crozier develops, the last three cells of an ascogenous hypha contain only nuclei which carry *I*. On the other hand, ascospores with only *I* nuclei germinate and form branched germ tubes with a few cross walls before they die. It may be that occasionally a mating *AiD* \times *aiD* would develop ascocarps, the asci of which would be homozygous for both lethals, especially where races like ($G5.3 \times C8$) 2.11 and ($481.13 \times C4$) 10 were mated and where *aiD* and *AiD* nuclei are proved to be present, although relatively inactive in sexual reproduction.

NON-HERITABLE INDURATED ASCUS ABORTION.—In the paper (Dodge, 1934) in which the race bearing the lethal *I* was first described, mention was made of a certain unknown environmental condition which on three occasions had affected ascus development in such a way that many asci became indurated in a manner exactly comparable to the indurated asci due to the heritable factor *I*. In each case attempts were made to propagate the races which developed these indurated asci by making transfers to fresh media and by germinating ascospores which were not infrequently developed in the same ascocarps. In no case was it found possible to preserve this condition in culture. This proved that the effect was purely a temporary and non-heritable one.

During the spring of 1937, while additional experiments were being carried out as a continuation of the work which had just been reported (Seaver, 1937), the same type of indurated asci appeared spontaneously in cultures, this making the fourth time in twelve years. This time, however, induration occurred in connection with fifteen cultures, eight of which were of matings which would have given asci heterozygous, *AaiiDd*, for the recessive lethal *d* and which, therefore, should have cut out four spores normally in each ascus (fig. 7, A). All of these cultures showed perithecia with a large proportion of indurated asci such as are shown in figure 7, A'. The other seven cultures in the same crate were of matings of two races such that asci homozygous, *Aaiidd*, for the recessive lethal should have been formed, to give a picture comparable to that shown in figure 7, B. It was found, instead, that all of the ascocarps in these cultures produced only indurated aborted asci (fig.

7, B'). As no work with the race carrying the dominant lethal *I* was being done at that time, it was suspected that the condition described above was again due to the peculiar environmental effects encountered on the three previous occasions noted above. Several sub-cultures were made, spores from cultures (see fig. 7, A') were also germinated, and additional cultures were obtained from single spores. In all cases the results proved that the effect had again been purely a temporary non-heritable one. All of the sub-cultures, as well as those from single ascospores, now produced only asci with four spores and no ascus abortion. Crushed mounts now gave a picture similar to that shown in figure 7, A, just as they should have done originally. Sub-cultures from the seven cultures giving total indurated abortion, such as shown in figure 7, B', produce asci, all of which aborted as shown in figure 7, B.

Where the matings were such that asci were heterozygous, *AaiiDd*, the environmental effect was the same as the genetic effect of the dominant lethal, *I*, in heterozygous asci, *AaiiDd*, with many asci indurated but always with some other asci cutting out spores. When matings were such that all asci were homozygous, *Aaiidd*, for the recessive lethal for deliquescent abortion, all asci had to abort with no spore formation whatever, the environmental effect being superimposed on the genetic deliquescent abortion effect. This would result in all asci that persisted becoming indurated. While indurated asci can now be produced at will by selecting one parent which carries the factor, *I*, we are still ignorant of the nature of the peculiar environmental condition which is capable of causing the non-heritable type of induration.

SUMMARY

Bisexual races of *Neurospora tetrasperma* in which the nuclei of one sex carried the so-called dominant lethal, *I*, for indurated ascus abortion were crossed with unisexual tester races carrying the recessive lethal, *d*, for deliquescent ascus abortion. Bisexual haploid *f*₁ progeny which now carry both *I* and *d* in the same nuclei were selected for back-crossing with the recessive testers. In this way self-fertile bisexual races were obtained which produced only ascocarps in which all asci aborted without any spore formation whatever, and asci that persisted became indurated. All asci formed by these back-cross races were homozygous for the recessive, *d*, and heterozygous for *I*. These two types of lethal factors apparently segregate independently in regular order and react without serious interference, each in its own way in producing abortion so that their effects are additive. These new races furnish very desirable material for further study of linkage relations, possible reversion of *I*, and nuclear behavior in various kinds of aborting asci.

The behavior of nuclei bearing the dominant lethal, *I*, in their relations to nuclei not bearing *I*, in nuclear migration, mycelial growth and sexual reproduction is shown to be well worth further study.

Another instance of the occurrence of a non-heritable type of indurated ascus abortion exactly comparable to the heritable type is again reported. A certain unknown environmental condition so affected the cultures in one series as to result in complete induration of all asci that should have shown only the deliquescent type of abortion because they were homozygous for the recessive lethal. Asci in other cultures which were heterozygous for the lethal, *d*,

and therefore should have produced 4-spored asci without abortion, for the most part did abort, and they also became indurated, in this way again showing that the environmental influence has exactly the same effect as the lethal *I* except that it is not heritable.

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PHOTOSYNTHESIS AND THE ABSORPTION SPECTRA OF PLANT PIGMENTS. II¹

G. Richard Burns

THIS PAPER is the continuation of an effort to see how well a rather oversimplified conception of the absorption of radiation by a higher plant could be used to explain the amounts of photosynthesis at different wave lengths. It is with considerable trepidation that we venture into this field, since studies must have been made by other investigators with better apparatus, more suitable material, and more time at their disposal for investigation. The only conclusions to be drawn from the fact that nothing has been published are either that no positive results have been obtained or that results such as those presented in the present paper were not considered sufficiently conclusive. However, considering the state of published knowledge in this field, it would seem as though even this rough survey is a distinct contribution. The value of one result of this work, the determination of the approximate curve for the amounts of photosynthesis in different parts of the spectrum, was much diminished by Hoover's paper (1937) which gave a very accurate and more complete curve based entirely on direct experimental evidence (fig. 2A).

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I should like to thank Prof. Charles J. Moore, of the Chemistry Department, Hunter College, for taking over all my duties there prior to the opening of classes each Fall in order that I might have more time for this work. I wish also to thank John L. Reinartz of the Radio Corporation of America for advice on vacuum tubes and F. H. Shepard, Jr., of the same company for designing and checking the light measuring apparatus.

The more important aspect of the problem, the explanation of this curve in terms of absorption, was not greatly affected, although Hoover's values for photosynthesis at 660 and 440 m μ might be interpreted to indicate a slightly higher quantum yield in the violet than in the red, while in this work, perforce, a constant quantum yield has been assumed.

APPARATUS.—It will not be necessary to remind persons skilled in the use of the spectrometer that the wide slit openings used in the spectrometric determinations in this work covered a much wider range of wave lengths than is customary. In absorption and emission spectra most of the readings were made with a front slit opening of 0.030" and a rear slit opening of 0.040". These values corresponded to 28 and 37 m μ at 700 m μ , one half of this at 566 m μ , and double it at 880 m μ . The non-symmetrical opening of the slits, +3 m μ at 700 m μ , slit curvature, -2 m μ , and all other errors incident upon the spectrometer itself were ignored. It was felt that the readings were sufficiently accurate for exploratory work. For more accurate absorption spectra the photoelectric light intensity comparison apparatus of Shepard (1937) was employed. This was sensitive to a radiation of 40×10^{-6} microwatts per cm.² and maintained this sensitivity practically independently of the total light intensity. In cases where the entire available radiation could be placed on a thermopile, this instrument was still over one hundred times as sensitive. It required only alternating current, came to equilibrium in half an hour after being turned on, kept its zero

point for hours at a time, and had no perceptible lag in response. It cost only a few dollars and was constructed by the author, who was totally inexperienced in vacuum tube work. With photoelectric cells which had a maximum sensitivity at 830 m μ , dropping to half this at 950 and 577 m μ , 0.5 m μ slits could be used on an F 16 spectroscopic. For a reading device a light and calibrated shutter were used on one tube and the spectrometer and solutions on the other. The readings made with this instrument were not made at sufficiently close intervals to locate minor irregularities in the absorption curves.

culated efficiency in table 2 is the efficiency of the red light as compared to yellow. This last was obtained by multiplying the sodium efficiencies by 1.23, the efficiency of the sodium as compared to yellow (Burns, 1936). The odd numbered trees were grown in blue light and the even numbered in red light; the radiation intensity is given, in this set of experiments only, in calories per cm.² \times 10. The second tree used in these determinations was a Norway spruce, and these values were included, since with a different light in each experiment a duplicate determination was necessary to show the accuracy of the

TABLE 1. The reflection of the needles and transmission of a solution of the pigments from a white pine grown in blue light. Also the spectra of the lights used in the photosynthesis experiments.

Wave lengths, m μ .	1223	1061	938	828	752	693	646	608	577	550
"Blue" pine 1										
% reflection	40	50	73	76	52	10.8	11.6	13.7	17.2	19.3
% transmission	85	95	95	96	94	44.0	6.8	14.5	27.2	40.0
Yellow light							10.0	45.5	49.0	4.0
Red light run no.										
15-20	17	115	119	290	345	167	1	0		
37	2	17	24	158	310	274	68	2	0	
28-39	2	15	22	171	336	233	8	0		
40	2	15	22	166	328	230	11	0		
41	2	15	22	146	299	226	15	0		
42	2	15	22	186	374	280	16	0		
43	2	15	22	172	348	235	7	0		
44	2	14	20	150	296	117	0			
45		13	16	156	324	172	2	0		
46		12	16	143	313	206	4	0		
47		11	18	158	324	192	2	0		
52	14	108	114	292	335	128	2	0		
53	12	119	129	342	416	214	3	0		
54	11	103	117	329	400	199	2	0		
55	10	93	106	312	393	191	2	0		
58	11	86	96	292	375	185	2	0		
59	14	93	111	336	432	245	6	0		
60	16	82	105	320	417	255	13	0		
61-62	17	95	118	391	510	312	12	0		

EXPERIMENTAL DATA AND DISCUSSION.—The most severe test of the calculation of relative amounts of photosynthesis from the "primary absorption spectrum" of a plant (see summary) was the following experiment in which one of the portions of the spectrum was largely in the infra red with the result that the calculated photosynthesis depended to about 80 per cent on the readings at 693 m μ . The light sources were 2000 watt, 200 hour incandescent lamps plus 25 mm. of a 7.4 per cent or 13.8 per cent solution of ferrous ammonium sulfate plus 13 mm. of a 0.5 per cent solution of brilliant scarlet 3R to which had been added 5 to 25 cc. of acid green L, 2 g. per liter. These two types of lights were designated as "7" and "14," respectively, and the spectra are given in table 1 and figure 1. For the standard light sodium lamps plus 20 mm. of water were used. The cal-

figures for the pine. The reflection and transmission spectra for spruce and hence the calculated efficiencies were nearly the same as for pine. The reflection and transmission for P1 in this series were determined (table 1), but for "red" pines the figures from an earlier paper (Burns, 1937) were used.

Disregarding physical measurements entirely, these experiments show that visible radiation is not necessary for photosynthesis since the "7" radiation contained almost no visible. The filaments of the 2000 watt bulbs could just be seen through the filters. A high sensitivity photronic cell gave a reading of 7 in this radiation as against 100 in a sodium light causing the same amount of photosynthesis. It should also be noticed that the large differences in the amounts of infra-red radiation beyond 800 m μ seemed to be without effect.

TABLE 2. The first column is the serial number of the day on which the experiment was performed, July 1 being 1. The second is the tree used, P for white pine and S for Norway spruce; even numbers indicate trees grown in red light and odd, in blue light. The meaning of the symbol for the lights used is given in the text. The carbon dioxide decrease is the average of two determinations which seldom differed by as much as two in the last place and is in percentage by volume. The total volume was five liters. The radiation intensity is the total radiation in calories per sq. cm. per run multiplied by the factor given. The last line for each tree is the dark respiration, the average of the projected areas from the position of each light, and the wet weight of the needles.

Run	Tree	Light	Carbon dioxide decrease %	Radiation intensity cal. $\times 10$	Efficiency of red	
					Obs.	Calc.
37	P1	Na	0.217	22.55		
		14	0.342	79.4	0.50	0.43
38		14	0.243	95.2	0.33	0.31
		Na	0.140	17.25		
39		Na	0.227	25.45		
		14	0.161	80.4	0.30	0.31
40		14	0.216	91.3	0.32	0.32
		Na	0.223	24.45		
41		Na	0.225	24.6		
		14	0.220	90.3	0.33	0.34
42		14	0.244	93.0	0.33	0.33
		Na	0.231	24.05		
		Dark	-0.077			
37	S1	Na	0.188	23.15		
		14	0.286	79.7	0.49	
38		14	0.176	95.5	0.32	
		Na	0.118	19.1		
39		Na	0.186	26.7		
		14	0.115	75.1	0.32	
40		14	0.165	89.3	0.32	
		Na	0.189	25.0		
41		Na	0.191	25.25		
		14	0.172	91.2	0.32	
42		14	0.193	93.6	0.33	
		Na	0.186	24.3		
		Dark	-0.076			
58	P1	7	0.092	141.2	0.14	0.18
		Na	0.200	24.95		
59		Na	0.208	25.70		
		7	0.146	145.2	0.17	0.20
60		7	0.230	148.3	0.22	0.22
		Na	0.225	26.55		
61		Na	0.226	25.7		
		7	0.218	140.8	0.22	0.23
62		7	0.229	130.7	0.24	0.23
		Na	0.220	24.85		
		Dark	-0.088			
58	S1	7	0.072	133.4	0.16	
		Na	0.159	25.85		
59		Na	0.162	25.85		
		7	0.096	142.3	0.17	
60		7	0.180	144.9	0.23	
		Na	0.165	25.60		
61		Na	0.175	25.65		
		7	0.170	139.7	0.22	
62		7	0.165	125.6	0.23	
		Na	0.175	24.70		

TABLE 2 (continued)

Run	Tree	Light	Carbon dioxide decrease %	Radiation intensity cal. $\times 10$	Efficiency of red	
					Obs.	Calc.
		Dark	-0.093			
15	P2	7	- .008	178.2	0.10	0.20
		Na	0.021	17.5		
16		Na	0.048	17.8		
		7	0.082	173.6	0.15	0.20
17		7	0.055	153.2	0.15	0.20
		Na	0.090	22.85		
18		Na	0.094	23.7		
		7	0.028	147.6	0.12	0.20
19		7	0.107	175.7	0.16	0.20
		Na	0.118	23.35		
20		Na	0.101	23.10		
		7	0.135	182.3	0.18	0.20
		Dark	-0.106			
15	S2	7	0.072	171.3	0.15	
		Na	0.049	18.2		
16		Na	0.052	18.55		
		7	0.125	161.0	0.22	
17		7	0.109	142.0	0.20	
		Na	0.112	23.63		
18		Na	0.115	24.15		
		7	0.060	143.4	0.15	
19		7	0.139	171.0	0.17	
		Na	0.137	23.35		
20		Na	0.132	23.35		
		7	0.173	172.9	0.20	
		Dark	-0.092			
43	P2	Na	0.113	24.2		
		14	0.156	85.5	0.40	0.42
44		14	0.008	69.0	0.21	0.32
		Na	0.111	23.35		
45		Na	0.129	25.45		
		14	0.093	74.8	0.35	0.38
46		14	0.149	78.9	0.42	0.43
		Na	0.133	25.2		
47		Na	0.137	25.65		
		14	0.124	79.6	0.37	0.40
		Dark	-0.090			
43	S2	Na	0.113	24.25		
		14	0.176	84.3	0.47	
44		14	0.014	67.9	0.23	
		Na	0.112	24.0		
45		Na	0.137	25.8		
		14	0.109	75.2	0.37	
46		14	0.168	80.4	0.44	
		Na	0.138	25.25		
47		Na	0.138	25.45		
		14	0.141	80.5	0.40	
		Dark	-0.090			
52	P4	Na	0.193	25.30		
		7	0.073	126.0	0.14	0.22
53		7	0.218	149.7	0.24	0.27
		Na	0.188	25.65		

TABLE 2 (continued)

Run	Tree	Light	Carbon dioxide decrease %	Radiation intensity cal. $\times 10$	Efficiency of red	
					Obs.	Calc.
54		Na	0.171	25.60		
		7	0.142	145.0	0.19	0.26
55		7	0.188	147.3	0.23	0.27
		Na	0.167	25.95		
		Dark	-0.072			
52	S4	Na	0.106	25.55		
		7	0.030	122.9	0.15	
53		7	0.131	149.0	0.22	
		Na	0.124	25.85		
54		Na	0.099	25.80		
		7	0.099	144.3	0.22	
55		7	0.111	146.0	0.21	
		Na	0.121	26.20		
		Dark	-0.071			
				Radiation intensity cal. $\times 36.5$	Efficiency of HgCn	
					Obs.	Calc.
57	P7	HgCn	0.121	71.4	0.980	0.92
		NaCu	0.141	78.9		
61		NaCu	0.133	78.2		
		HgCu	0.105	68.8	0.953	
62		HgCu	0.115	66.9	1.005	
		NaCu	0.139	77.4		
63		NaCu	0.133	77.4		
		HgCu	0.088	63.0	0.909	
		Dark	-0.041	: 98 cm ² .	: 8.01 g.	
57	P8	HgCu	0.063	73.2	0.869	0.91
		NaCu	0.083	74.0		
61		NaCu	0.076	75.9		
		HgCu	0.059	73.5	0.903	
62		HgCu	0.061	67.8	0.940	
		NaCu	0.078	73.2		
62		NaCu	0.075	75.0		
		HgCu	0.050	63.0	0.970	
		Dark	-0.059	: 96 cm ² .	: 8.61 g.	
65	P9	Hg	0.266	125.4	1.073	0.93
		Na	0.221	117.0		
66		Na	0.235	116.4		
		Hg	0.255	130.4	0.949	
67		Hg	0.259	132.8	0.952	
		Na	0.237	118.2		
68		Na	0.238	118.0		
		Hg	0.261	127.2	0.995	
		Dark	-0.081	: 131 cm ² .	: 10.73 g.	
65	P10	Hg	0.187	121.6	1.090	0.89
		Na	0.148	114.4		
66		Na	0.154	114.6		
		Hg	0.180	122.4	1.03	
67		Hg	0.187	123.0	1.01	
		Na	0.165	114.8		
68		Na	0.169	115.4		
		Hg	0.191	119.4	1.04	
		Dark	-0.102	: 173 cm ² .	: 14.66 g.	

Taking from table 2 averages of the more accurate values—experiments in which the apparent photosynthesis was about the same—the following figures are obtained:

Tree	Observed	Calculated
P2	0.16	0.20
P1	0.23	0.23
P4	0.22	0.27
P1	0.33	0.33
P2	0.40	0.42

There are two points which might be mentioned about these figures. If the primary absorption of the "red" pine at 752 m μ was lowered from 10 to 8.25 per cent to correspond to that of the "blue" pine (Burns, 1937), then the agreement between the calculated and observed values was much improved not only in these experiments but in all others in which this value was used. Also the fact that the difference between the "red" and the "blue" pines was in the yellow and not in the red—i.e., was due to changes in the relative concentrations of at least two pigments—may be shown by calculating the efficiency of a "red" tree, basing the primary absorption curve on the absorption of "blue" tree pigments diluted to the same absorption in the yellow. The efficiencies thus calculated were some 30 per cent too low. However, these are rather fine distinctions to make on such rough spectral measurements.

In this experiment the calculated amount of photosynthesis depended on the readings at 693 and 752 m μ , particularly on the 693 values. Since the wide slits on the spectrometer lowered the peaks of the curves and widened the bases, and since, in this case, the two curves intersect near the middle, the spectrometer errors due to the wide slits tend to cancel out. If the readings on the lights had been made with narrower slits, the slope of the primary absorption curve would have to have been steeper to get a check.

There is one additional point which should be mentioned. The value for the primary absorption, with wide slits, at 752 m μ was several times greater with these pines than was found with other simpler plants or for pines which had been grown in filter houses at higher temperatures. While no experiments were performed on these plants, it seemed rather improbable that they would differ as much photosynthetically as their calculated values indicated. In other words, the difference between the primary absorption curve and the photosynthesis curve at this point may be greater than these results indicate.

In one of the earlier experiments (Burns, 1936) the radiation from sodium lamps was found to have an efficiency of 1.23 when compared to yellow radiation from a filtered incandescent source (table 1). The calculated efficiency of the sodium was about 1.05. The high observed value may be due, in part, to the fact that this source was about one hundred times the size of the incandescent light and so illuminated the plant more uniformly, but was probably due to the fact that this is an intermittent source (Emerson and Arnold, 1932; McAllister, 1937; etc.). This light

flashed 120 times a second with the change of intensity of each flash following, very roughly, a rectified sine curve (Found, 1934) so that the dark period was relatively short. The sixteen per cent increase in efficiency could probably be accounted for on this basis. Since this effect would approach zero with decreasing light intensity, care was taken to use the sodium lights at this intensity when they were being compared with a steady source. Arthur and Stewart (1935) found the sodium lamp particularly efficient in dry weight production but concluded, "From the data of Zscheile the absorption at wave length 588 is less than 10% Chlorophyll absorption has very little if any relation to the efficiency of a band of energy in the production of dry weight increases in buckwheat plants." Since Zscheile (1934) published this part of his data as a graph, it is rather difficult to determine the values for the absorption coefficient, but they are about seven for both chlorophyll a and chlorophyll b. Using Arthur and Harvill's data (1937) for the percentage of chlorophyll and the ratio of a to b in buckwheat and assuming an area of 100 cm.² per gram, the probable minimum absorption would be about 35 per cent at 589 m μ . The primary absorption of white pine was found to be 40–60 per cent. It would appear that they have assumed too low a concentration of chlorophyll to

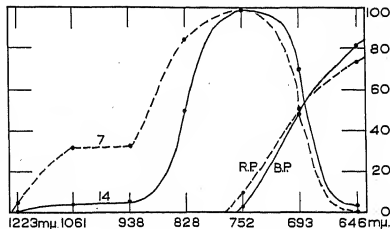


Fig. 1. Examples of "7" and "14" radiation and the primary absorption spectra of a red and blue white pine showing that the spectrometric errors due to the wide slits tend to cancel out in this case, and the photosynthesis curve for equal incident quantum intensities would be only slightly lower at 752 m μ , and the same or slightly higher at 693 m μ .

represent conditions obtaining in their plants and also failed to consider the fact that the source was intermittent. Geo. P. Burns (unpublished work), in experiments that were similar except that the radiation from the incandescent source was filtered through 30 mm. of water and the buckwheat plants were exposed for 18 hours a day for much longer periods, found that the two radiations always resulted in practically the same dry weight increase, height of plant, color, and general appearance. The main difference in the two experiments was the large amount of infra red present in Arthur's incandescent light. We have found that large amounts of infra red interfere with

photosynthesis (1937) and induce elongation. Since the purpose of this experiment was to see whether intensities of these lights which gave the same calculated amount of photosynthesis with white pine might be expected to give similar results with other plants, the fact that they gave the same reading on a photronic cell was entirely accidental.

Since line sources would be very much more suitable for this type of work, two preliminary experiments were carried out with them, more with an idea of determining how much changing certain variables would affect the results than for the purpose of obtaining definite information on the primary absorption spectra. In these experiments (table 2) the yellow sodium line was compared with the yellow mercury line. The designations of the lights and the filters were:

Source	Filters	Designation	Approximate fraction of radiation in yellow line
Sodium	30 mm. of 0.66% copper sulfate	NaCu	.93
Sodium	30 mm. of water	Na	.87
Mercury	20 mm. of water containing 5% potassium dichromate and 3% copper sulfate. (Unstable)	HgCu	.93
Mercury	20 mm. of 6% potassium dichromate	Hg	.90

Intensity and absorption measurements were made with an additional 10 cm. water filter, although this was not used on the plants. The reflection measurements were made for the main line only. The radiation intensities in table 2 are calories per cm.² per min \times 36.5, and the last line under each tree is the respiration, projected area, and wet weight of the needles. The mercury lamps were the "High Intensity" type operated on alternating current, and the light output follows a sine curve varying from 37 to 163 per cent of the average intensity (St. Louis, 1936). This was quite different from the sodium lamps.

PHOTOSYNTHESIS IN SHORT WAVE LENGTH RADIATION.—It is interesting to apply to the results in the violet end of the spectrum the idea that the amount of photosynthesis is determined by the primary absorption spectrum, together with the perhaps unjustified but necessary assumption that the quantum yield does not change. These efficiencies, while not particularly accurate, are so very much less than those obtained by many other investigators that the differences far exceed the experimental errors. Since in this region the yellow pigments of the plant begin to absorb radiation and it is not known whether or not this energy is then available for photosynthesis, the primary absorption spectrum was taken as the total absorption minus the fraction absorbed by the carotin and xanthophyll but not, because of experimental difficulties, the radiation absorbed by other yellow

pigments in the leaf. In the determination of the absorption spectra of the carotin-xanthophyll mixture, stray radiation in the spectrometer was cut down by a copper sulfate filter on the source. These data were obtained on pines from the red filter house only and were used for both types. Pines from the blue house showed an efficiency of about 0.68 (Burns, 1936) in the blue radiation as compared with the yellow, while the calculated efficiency on the above basis was found to be 0.64; the red pines were about 0.77, observed, against 0.71, calculated. If it were assumed that all the yellow pigments were effective

TABLE 3. Reflection, absorption, observed and calculated efficiencies for pines which had been grown in red and blue light, in the various filtered sodium and mercury lights.

Tree	Sodium		Mercury		Efficiency of mercury	
	Percentage reflected	Percentage absorbed	Percentage reflected	Percentage absorbed	Found	Calc.
P7	17	80.7	19	77.8	0.95	0.92
P8	19	66.8	22	64.0	0.93	0.91
P9	17	77.1	19	74.7	0.93	0.93
P10	19	68.2	22	64.4	1.03	0.89

Without certain unjustified assumptions, these experiments cannot be interpreted, but there are no spectrometric determinations, the quantum value of the two lights was nearly the same, and, if it is assumed that the difference in the types of light flashes is without effect, the experiments offer some confirmation (table 3) of the idea that the amount of photosynthesis depends on the primary absorption spectrum as the reflections vary from 17 to 22 per cent, the absorptions from 64 to 81 per cent.

in photosynthesis, the calculated efficiency of the red pine would have been 1.07. While these data leave much to be desired as to accuracy, it would appear that radiation absorbed by the yellow pigments was not wholly available for photosynthesis. Warburg at one point explained his results by assuming that 30 per cent of the light absorbed by carotin and xanthophyll was used. Between 500 and 400 m μ the curve would thus differ markedly from Hoover's (fig. 2A). Since this area was covered in one experiment, it

was not possible to draw conclusions as to the actual shape of the curve, and, since half the radiation was longer than 500 m μ , the results are not very accurate; nevertheless, the total amount of photosynthesis in this region was not as great as that found by Hoover, and it does not seem possible that the curve could have been as high as his, which, on an incident quantum basis, rises to a maximum at 400 m μ higher than the one at 660 m μ . Our curve, however, could have the same general form as his by considering the absorption by yellow pigments other than carotin-

xanthophyll as ineffective and allowing partial effectiveness to that absorbed by the carotin-xanthophyll.

THE QUANTUM YIELD.—The quantum yield, using the primary absorption spectra for the absorbed light, was about 9.5 quanta per molecule of carbon dioxide, for the green and orange light and 8.0 quanta for the sodium and mercury; a rough check, at least, of the higher efficiency of the intermittent lights.

The "primary absorption spectra" given in this paper are not, of course, the absorption spectra of the plant, nor are they, because of the wide slit openings used, the true primary absorption spectra. It would be difficult to determine the absorption of a pine needle, but figure 2B gives the absorption spectrum, 90° reflection and transmission, of a maple leaf (a) as compared to its primary absorption spectrum, 45° reflection, made with narrow slits (b). The valves for the leaf depend on a roughly measured factor for the ratio of total light transmitted to the transmitted light measured. Figure 2C is also for a maple leaf and shows the transmission of the pigments in solution with narrow slits (c), with wide slits (a), and that of the leaf itself multiplied by an arbitrary constant, about twice the actual constant, to give 95 per cent transmission at 752 m μ . Apparently the true absorption of a leaf is somewhat higher in the red than the primary absorption even when the latter is measured with wide slits. Since photosynthesis was also higher, this might be interpreted to mean that photosynthesis depends not on the absorption spectrum of chlorophyll but on the spectrum as modified by the condition of chlorophyll in the leaf. The difference between these curves in the middle of the spectrum may be without actual significance, as very little of the reflection of a leaf is to light of 90° incidence, although it would appear that photosynthesis in this region was below the true absorption.

SUMMARY

The relative amounts of apparent photosynthesis in two portions of the spectrum, one usually in the yellow, were determined for white pine trees grown in red or in blue-violet light. There were rather large changes in the relative amounts of photosynthesis with the same tree from day to day in a given set of lights, the cause of which has not been determined and so must be considered an experimental error. The spectra of the sources were determined with very wide slits on the spectrometer as were the reflection spectra of the plants to light of 45° incidence and the absorption spectra of an 80 per cent acetone solution of the plant pigments at the same concentration as in the plant. The absorption curve determined in this manner was called the primary absorption spectrum of the plant. Using these figures and assuming a constant quantum yield, the calculated amounts of photosynthesis were determined and found to agree within a few per cent with the experimental values at wave lengths longer than 500 m μ . Thus the primary absorption spectrum and the photo-

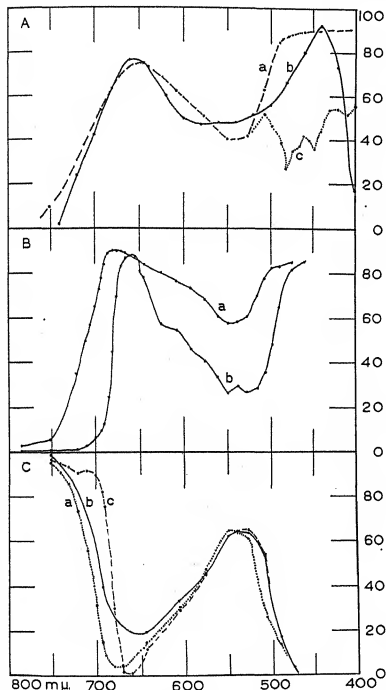


Fig. 2.—A. Hoover's curve for photosynthesis with wheat on an equal incident quantum basis (b). Primary absorption spectrum or photosynthesis at equal incident quantum intensity for a white pine grown in red light (a). Primary absorption spectrum minus light absorbed by carotin and xanthophyll (c).—B. Approximate absorption spectrum of a maple leaf to light of 90° incidence (a). Primary absorption spectrum of same leaf determined with narrow slits (b).—C. Percentage transmission of incident light by maple leaf multiplied by two (a). Transmission of pigments in solution at same concentration with wide (b) and with narrow (c) slits.

synthesis curve for equal incident quanta intensity were the same in this region. While the difference in the primary absorption spectra between trees grown in red and in blue light seemed to result in a corresponding difference in the amounts of photosynthesis, there was no evidence to show that this is a general relationship even with white pine, as these experiments included no cases of extreme differences in color. The differences in color between the red and the blue pines were due to the changes in the ratios of the concentrations of at least two pigments.

Correcting these data for spectrometric errors due to the wide slits, the primary absorption curve is about the same as the photosynthesis curve from 680

to 520 m μ , photosynthesis is slightly less at 752 and slightly greater at 693 m μ , and shorter than 520 m μ , much less. A possible explanation for the similarity between the photosynthesis and the primary absorption is that the primary absorption curve was rather close to the actual absorption of the leaf, much closer than the absorption spectrum of the pure chlorophylls.

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CYTOLOGY OF LEAVES AFFECTED WITH LITTLE-LEAF¹

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PLANTS AFFECTED with functional disorders caused by deficiencies of one sort or another often exhibit brachytic shoots and dwarfed leaves. The general unthrifty appearance due to the dwarfing has received a variety of names among which rosette, little-leaf, mottle leaf, and yellows may be mentioned.

The interveinal areas of affected leaves are often chlorotic, as indicated in figure 1, especially if the leaves grow in positions where they receive strong illumination. The dwarfness is due to inhibited growth rather than a reduction in the fibrovascular system, since the number of lateral veins in affected leaves is the same as that in the healthy. The affected leaves of apricot and peach are often so brittle that wind whipping breaks them when they strike other parts of the branch. Orange leaves are conspicuously dwarfed but are not fragile (Reed and Parker, 1936). The depauperate condition of rosetted pecan leaflets

results in a crinkled or undulated condition of the lamina (Rand, 1922), and in acute cases the leaflet may consist only of the midrib bordered by an edging of ragged tissue.

Examples of little-leaf in various other plants grown under experimental conditions have also been given by Hoagland, Chandler, and Hibbard (1936), who demonstrated that in severe cases of the disease, the leaves of the squash and cotton may, in addition to nanism, have necrotic spots. The symptom on maize called "white bud" also belongs to this category (Barnette and Warner, 1935).

Evidence now available shows that small amounts of zinc salts properly applied have a specific effect in alleviating the dwarfed, chlorotic, depauperate character, not only of the leaves, but of the shoots (Chandler, Hoagland, and Hibbard, 1932, 1933; Johnston, 1933; Finch and Kinnison, 1933). The amount of zinc applied in these cases is extremely small, yet its effect is tremendously beneficial to the plant concerned. It seems plausible to assume that it acts as an activator of some sort which promotes the growth

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processes and maintains the physiological integrity of the organism by indirect means. Hoagland, Chandler, and Hibbard (1936) proved that absence of chlorophyll in the leaves of affected plants was not due to deficiency of soluble iron.

Chandler (1937) has advanced an important idea concerning the role of zinc in carbohydrate metabolism. In view of the fact that zinc deficiencies are more acute in plants where carbohydrates have obviously accumulated, it seems probable that some essential process of carbohydrate transformation is inhibited. This idea is corroborated by observations to be reported in this paper showing that in the zinc-deficient plants there are serious derangements in the constitution of the assimilating cells of affected leaves.

The zinc content of orange leaves appears to bear a certain relation to the amount of chlorophyll they contain and to migrate towards the most physiologically active cells, such as those found in the leaf parenchyma and the undifferentiated cells of the meristem (Reed and Dufrenoy, 1935b).

The purpose of this study was to investigate the cellular conditions of plants affected with little-leaf and to compare them with similar plants in which there was no evidence of malnutrition. The results, though generally qualitative in nature, have thrown light on some important phases of the problem and supplement those obtained by other methods of attack. The cell being the fundamental unit of the organism, it is logical to consider the changes and derangements which occur there.

The investigation is based on the study of leaves of apricot, peach, tomato, maize, squash, mustard, and buckwheat. Although certain phases of cell growth are necessarily discussed, there was no effort to follow the complete ontogenetical development of the cells. The materials for study were obtained both from the orchard trees and from controlled experiments in soil and in nutrient solution.

THE GROWTH AND MULTIPLICATION OF LEAF CELLS.—In the early stages of development of apricot leaves, as found in the San Joaquin Valley of California prior to March 10, there was little difference in the cytology of healthy and affected leaves. The young parenchyma cells of the embryonic leaves contained large nuclei and finely vacuolated cytoplasm (fig. 2). The cells of the palisade parenchyma at that time were rhomboidal and multiplied in a manner which seemed essentially similar in affected and in healthy leaves. Their meristematic condition was also indicated by their staining reactions. A few mitochondria had begun to develop into plastids. Precipitates of phenolic material were present in the epidermal cells of both the affected and the healthy leaves.

The form of cells in embryonic peach leaves at corresponding dates was somewhat different. The palisade cells of healthy leaves (fig. 5) were rhomboidal, similar to those of apricot leaves already described, but those of the affected peach leaf were smaller and less differentiated.

Retarded differentiation is characteristic of the little-leaf disease, illustrated by the development of rhomboidal instead of columnar palisade cells in affected apricot leaves collected April 20 at Delhi, although at that time the contents of the cells showed scarcely any signs of disorganization. The mesophyll tissue was conspicuously compact, also suggesting that the juvenile stages were prolonged.

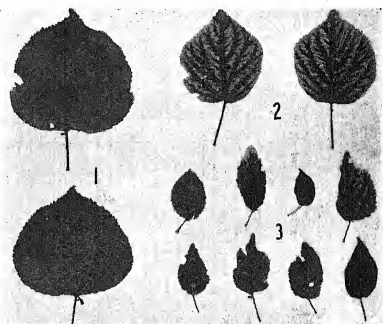


Fig. 1. Mature apricot leaves from orchard trees. 1, unaffected; 2, affected with little-leaf indicated by mottling and dwarfing; 3, affected with little-leaf indicated by severe mottling and brittleness (broken margins).

Hypertrophy of the palisade parenchyma and atrophy of the mesophyll were well shown by a comparison of transverse sections of healthy and affected tomato leaves grown in Delhi soil under experimental conditions in the greenhouse. The characteristics of this soil and the depauperate character of the plants produced therein have been described by Hoagland, Chandler, and Hibbard (1936). The tomato leaf sections shown in figure 3 were from material fixed, embedded, and stained by the same methods. The drawings were made under the same magnification with the aid of a camera lucida. The palisade parenchyma cells of the affected leaves were larger than those of the unaffected leaf, though the total number was smaller, indicating that one effect of the disease is promotion of cell growth, rather than cell multiplication. There was also well marked atrophy of the mesophyll.

The chlorophylliferous cells of the maize leaf, although different in shape, nevertheless show hypertrophies in leaves of plants grown without the addition of zinc. This is especially evident in plants which have shortened internodes and show the "white bud" effect.

As noted elsewhere in this article, the cell structure of affected leaves of certain plants is singularly compact, especially in the part of the leaf occupied by

the mesophyll (fig. 4), suggesting a xerophytic type of leaf. Apricot, peach, walnut, and sunflower leaves showed this type of development more than mustard and squash. This compactness was found at a comparatively early date (June 12) in the leaves of apricot seedlings grown in a culture solution lacking zinc. Although mottled, the leaves were not dwarfed, yet the symptom was evident. Leaves from affected apricot trees collected from the orchard in July showed extremely compact mesophyll.

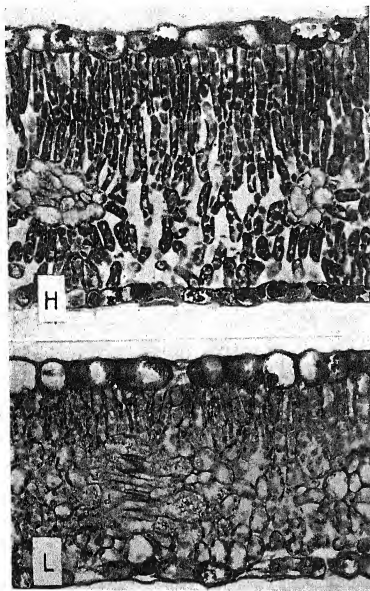


Fig. 4. Effect of little-leaf disease on the structure of peach leaves. H, healthy; L, affected with little-leaf, both magnified 300 diameters.

Reed and Dufrenoy (1935a) described a similar phenomenon in mottled orange leaves and called attention to prolongation of the juvenile leaf structure. Rand (1922) has previously demonstrated similar exaggerated growth of palisade parenchyma cells in the case of pecan rosette, also accompanied by lack of differentiation. He found that there was a reduction in the size of intercellular spaces within the spongy parenchyma resulting, in some cases, in almost complete obliteration.

The tremendous reduction in intercellular spaces may result in diminished gaseous exchanges in the affected leaves, which means that the cells containing

plastids may obtain only a small part of the substances necessary for the synthesis of carbohydrates or for respiration.

The little-leaf disease thus appears to affect similarly a fairly wide range of plants.

THE PROTOPLAST.—The protoplast fills the cell cavity in embryonic leaves with cytoplasm and living inclusions such as nucleus and mitochondria. Differentiation leads to specialization and vacuolization.

In the embryonic stage there is no observable difference between affected and healthy leaves except in their reaction to dyes. The protoplast of affected peach leaves shows hyperchromatization, indicated by figure 5. Since meristematic cells of these plants have comparatively little affinity for the dyes used in this cytotechnic, the increased affinity for stains is a very sensitive indicator of derangement of vital conditions. Hyperchromatization is characteristic of nuclei and cells in "silvered" plum leaves (Tetley, 1932).

Fresh sections of affected peach leaves collected at Delhi May 25 showed no evidence of polarization of the cell contents, though there were fat globules in the parenchyma cells which were stained with indophenol blue. After killing the leaves with boiling water and extracting the chlorophyll, a good test for starch could be obtained with iodine. The palisade and mesophyll cells absorbed neutral red sparingly, but the epidermal cells absorbed it freely.

The cells of affected peach leaves examined August 7 showed considerable disorganization and proteolysis especially in the palisade cells adjoining the dorsal epidermis, as shown by their strong reaction with Millon's reagent. Apparently the difference in illumination had an effect, since maximum disorganization was found near the surface of the leaf.

The cells of affected apricot leaves gradually lost their hyperchromatization as they grew out of the embryonic stage. Specimens collected in July from affected trees showed that proteolytic activities had reduced the cytoplasmic layers in most cells to an almost invisible film, although other organs, such as mitochondria, plastids, and nuclei showed comparatively no derangements.

The cells of unaffected apricot leaves collected in July showed no such extreme proteolysis. The parietal layers of cytoplasm were conspicuous, though thin, nuclei were apparently normal, and, although many mitochondria had developed into plastids, there were also many undeveloped mitochondria. Affected leaves contain more substances which react with ferrocyanide or Millon's reagent than healthy leaves. The reactions, though not specific, may indicate the protoplasmic degradation. Free-hand sections of leaves collected May 25 from affected apricot trees tested for proteins by the Loew-Bokorny method (1889) contained more blue-green material than leaves of corresponding age from unaffected trees. The blue-green color was most abundant in phloem cells and in the mesophyll. Control tests made it certain that the color developed in the affected cells was not due to tannin.

Investigations on living cells have shown that the contents of affected cells have a greater positive charge than the unaffected. For example, basic dyes like thionine, Nile blue sulfate, and Neutral Red penetrated the former more rapidly.

The polarization frequently seen in affected cells wherein the negatively charged plastids are assembled at the internal end of palisade parenchyma cells may support the hypothesis that the little-leaf disease increases the positive charge in some, if not in all, cells.

The greatest contrast in affected and healthy cells of apricot leaves was in the plastids, to which reference will be made subsequently. Mitochondria were present in the cells of affected apricot leaves collected in the orchard shortly before the leaves fell in November.

Most of the mitochondria in the phloem cells of affected corn leaves remained undeveloped and were readily demonstrated by the use of acid fuchsin and methyl green (Zirkle, 1929).

The nuclei of the epidermal cells of corn are large and are readily stained in sections of material fixed in Neme's solution. They were distinctly stained with safranin and light green or with aurantia and methyl violet. They were poorly stained with eosin, iron haematoxylin, or acid fuchsin and methyl green. The contents of the nuclei in healthy leaves were usually homogeneous, but in affected corn leaves they were either heterogeneous, due to the presence of chromatic granules, or indistinct. In cases where the leaf was not seriously affected by the "white bud" condition, the nuclear contents were homogeneous as in the healthy leaf.

The enzyme-secreting cells of *Drosera tentacles* (Huie, 1897) and cells of "silvered" plum leaves (Tetley, 1932) have shown that the stage of aggregation of nuclear material bears a certain relation to the vital processes of the cell.

THE CHLOROPLASTS.—There is need for still further investigation of the chloroplasts in relation to little-leaf disease. Many of the impaired functions of the leaves are obviously traceable to disrupted carbohydrate synthesis, as Rand (1922) indicated in his work on pecan rosette. Whether the factor which inhibits the development of mitochondria into plastids is also inimical to the existence of the full-sized plastid is yet to be determined.

Several lines of evidence support the idea that zinc salts promote the development and synthesizing functions of chlorophyll bodies in the leaves. Bertrand and Andreitcheva (1933) showed that green leaves of numerous plants were richer in zinc than etiolated leaves of the same varieties; Reed and Dufrenoy (1935b) demonstrated by a process of microincineration that there was more zinc in the cells of green than of mottled orange leaves.

Under the stimulus exerted by some plant parasites, the chloroplasts may respond by increasing the amount of chlorophyll. Beauverie and Cornet (1930) recorded this in the case of peach leaves infected with *Coryneum Beijerinckii*, where the plastids of the cells

adjacent to the infected area were not only deeper green, but their chlorophyll more resistant to the solvent action of absolute alcohol.

There are small dark green areas in the pale yellow tissue in the most extreme cases of mottling. Microscopical examination shows a corresponding development of chloroplasts even in cells surrounded by hypoplastic disorganized cells. These green "islands" indicate that the deleterious factors are not highly diffusible. The permanence of chlorophyll in the vicinity of the midrib and the principal veins is one of the most conspicuous features of the little-leaf type of disease.

Avocado.—The plastids are rather uniformly distributed in healthy palisade cells, but they often show agglutination in the cells of affected leaves resulting in a pronounced polarization of the cell contents, which is characteristic of hypoplasia. Figure 6 shows a case of extreme polarization in palisade cells of an affected avocado leaf, in which several plastids also show vacuolization of the stromata. This agglutination of the plastids is in no way due to plasmolysis of the cell contents because the tannin globules shown in figure 6 are not clumped and in other cases delicate cell structures showed no signs of displacement.

Corn.—Another example of the same thing in a very different plant is shown in figure 7. The illustration was made from a section of a corn leaf taken from a plant which showed symptoms of the "white bud," a zinc deficiency disease. Though vacuolization of the plastids had only begun, there was a pronounced clumping of plastids and a migration of the nucleus to the end of the cell.

Cytological studies were made on plants which showed dwarfing and chlorosis similar to the conditions described by Barnette and Warner (1935) and by Hoagland, Chandler, and Hibbard (1936).

The existence of two types of plastids (fig. 8, A and B) in the corn leaf presents some interesting phenomena. Amyliferous plastids occur only in the bundle sheath (Zirkle, 1929). In my material, the starch grains were enclosed in a coarse network of protoplasm. When a combination of acid fuchsin and methyl green was used, the amyliferous plastids were stained gray-green, and the nonamyliferous plastids were reddish purple. A combination of aurantia and methyl violet stained the former yellow and the latter violet, though neither color was permanent. Eosin stained nuclei and amyliferous plastids, but not the nonamyliferous plastids.

It will be noted from figure 7 that the number of plastids in affected corn leaves is materially reduced, although their size was not greatly altered. A leaf section bears considerable resemblance to "Argentea" leaves (Zirkle, 1929). Since the plastids in the fixed material did not readily absorb stain, their structure was not as evident as in apricot and other leaves.

In most of the corn leaves studied, the nonamyliferous plastids of the affected leaves showed vacuolization in the preliminary stages of degeneration (fig. 8B). In leaves which showed more pronounced

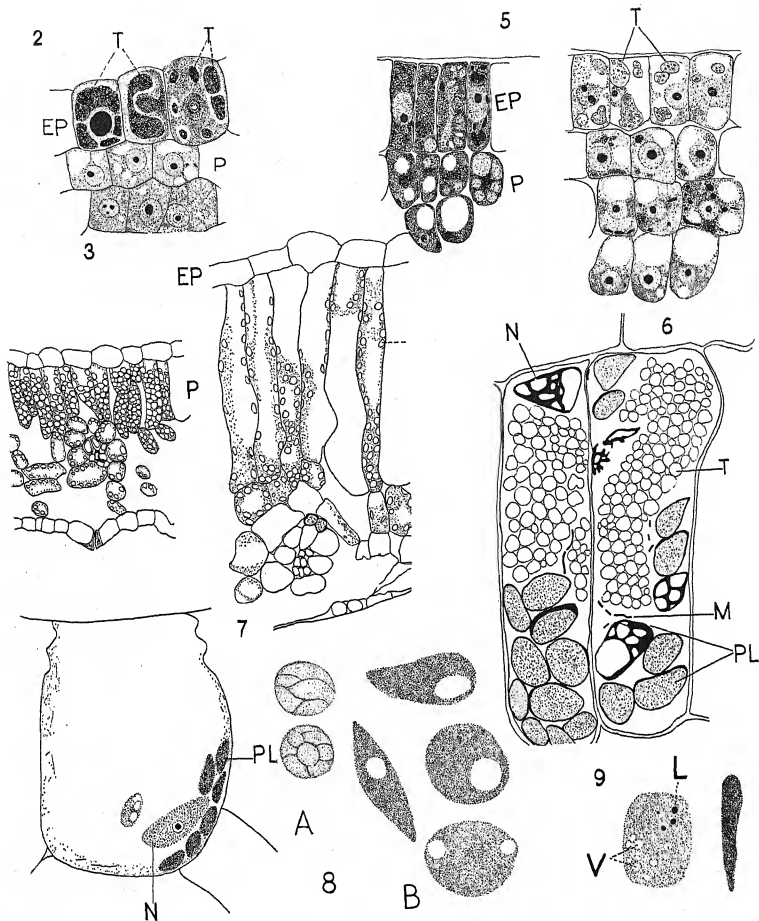


Fig. 2. Cells in an embryonic apricot leaf from an affected tree collected Feb. 29, 1936. The cells showed no serious derangement.

Fig. 3. Transverse sections of tomato leaves drawn on the same scale. The leaf section at the left was from a plant grown in Delhi soil which received zinc sulfate in addition to other nutrients (Ca, NO₃, K, PO₄). The leaf section on the right was made from a plant of the same age in the same soil receiving the same nutrients, but without zinc sulfate.

Fig. 5. Cells in embryonic peach leaves collected Feb. 29, 1936. Left, from an affected tree, showing hyperchromatization of the protoplasts; right, from a healthy tree, showing more advanced stage of differentiation and development of mitochondria. Both figures were drawn on the same scale of magnification.

Fig. 6. Palisade parenchyma cells from a mottled avocado leaf in which agglutination of the cell contents was evident. (Drawing by J. Dufrenoy).

"white bud" symptoms, the cells contained few plastids, reaching eventually the stage shown in figure 7. The vacuolization and agglutination phenomena are, therefore, more evident in the nonamyliiferous than in the amyliiferous plastids.

Plants seriously affected had dwarfed bronzed leaves. Fresh sections of such leaves showed great changes in the plastids as one might infer from macroscopic examination. The amyliiferous plastids (fig. 8A) contained starch, though the nonamyliiferous plastids showed serious disintegration of cell contents. After fixation in Nemece solution and staining with safranin plus light green, the finer details could be seen and were similar to those already described in the "white bud" symptoms.

Apricot.—The first development of chloroplasts in apricot leaves occurs in the mesophyll cells in the vicinity of fibrovascular bundles. The stages were plainly seen in very young, healthy leaves, which were plates of five to seven cells collected March 9 at San José. The young plastids stained readily with haematoxylin or acid fuchsin after fixation in Nemece's solution. Mitochondria in the young palisade parenchyma cells were also evident at that time in sections stained with haematoxylin. The cytological development in affected apricot leaves was essentially similar, though there was often a slight lag in the process.

The color of the young apricot leaves is due to anthocyanins which are removed by the reagents employed in dehydration. It is highly probable that tannins are the mother-substance of anthocyanins.

Collections of apricot leaves from healthy trees at San José on April 24 showed that the young leaves had reached a stage of full cytological differentiation. The leaves showed no symptoms of any type of derangement.

The palisade parenchyma cells were elongated and contained many plastids, each of which contained one to several starch grains. The nuclei were large and contained nucleoli which stained deeply and presented a contrast to the otherwise lightly stained nucleoplasm. The physiological activity of the cells was apparently at a maximum for the season. Precipitates of phenolic materials were absent from all cells except those of the bundle-sheath, where the amounts of the phenolic materials were very small.

The plastids of affected apricot leaves collected April 20 at Delhi generally contained large starch grains and showed no vacuolization or other signs of derangement, although there had been lysis in a few palisade parenchyma cells. None of the cells showed agglutination of plastids.

In the plastids of healthy apricot leaves collected July 29 there were no signs of disintegration, unless it were certain small granules of unknown nature

especially evident in material fixed in Meves' solution. The plastids were large and numerous, filling much of the cell space, yet they were generally devoid of starch granules, though the material was collected in the afternoon of a sunny day, and the fixation was made immediately. Starch grains, if present, were very small. The plastids stained readily with haematoxylin or with acid fuchsin and methyl green.

When comparative tests were made on fresh material, satisfactory evidence of starch was obtained, indicating that one must ultimately use the iodine tests on decolorized leaves. For example, apricot leaves collected at 11:30 a.m., May 26, 1937, from seedlings in the greenhouse at Berkeley showed no starch in the plastids of healthy or affected leaves when sections of fresh material were examined. However, starch was present in both samples of leaves, as demonstrated after killing in boiling water and removing the chlorophyll with alcohol. In the living plastid the iodine may not penetrate sufficiently to color the starch grains.

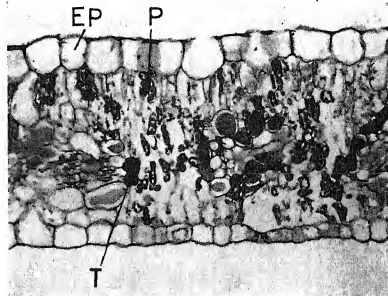


Fig. 10. Photomicrograph of affected apricot leaf in which extensive areas were cleared by lytic factors. $\times 150$.

The plastids (fig. 9) in affected apricot leaves collected July 29 showed both vacuolization and shrinkage. Droplets of lipid material (fig. 9L) in the stromata of the plastid were perhaps the first indications of derangement, followed by a transverse shrinkage in volume. Few plastids contained starch. The thin, fusiform plastids (fig. 9) of severely affected apricot leaves had a strong affinity for dyes, especially for acid fuchsin.

The lytic factors associated with this disease had destroyed many of the plastids and effectively "cleared" cells through wide areas (fig. 10) in apri-

Fig. 7. Palisade parenchyma cell from an affected corn leaf showing proteolysis and agglutination of the plastids.

Fig. 8. Plastids from corn leaves. Stained with iron alum haematoxylin.

Fig. 9. Plastids from affected apricot leaves collected July 29, 1936. Left, the plastid contained oil droplets and vacuoles; right, an affected plastid showing shrinkage. Fixed in Meves' solution, stained with iron alum haematoxylin.

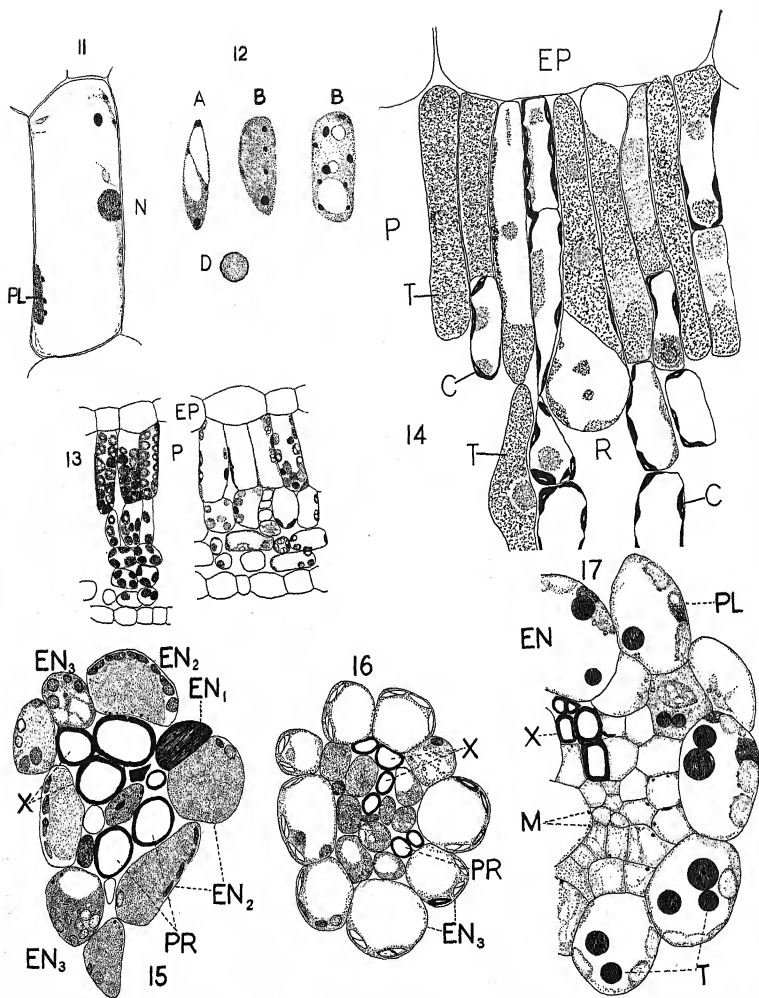


Fig. 11-17.—Fig. 11. Apricot leaf cell showing advanced stage of lysis in a leaf collected July 29.—Fig. 12. Plastids and sterinoplast from cells of affected peach leaves. Sample collected August 12 at Delhi, fixed in Meves' solution, stained with acid fuchsin plus methyl green.—Fig. 13. Sections of squash leaves. Left, portion of a section of a healthy leaf from a culture in soil to which zinc was added; right, portion of a section of an affected leaf from a culture in soil which received no application of zinc.—Fig. 14. Portion of a section of an affected apricot leaf collected Dec. 4 from a seedling tree grown in a nutrient solution lacking zinc. (Drawing by Dr. Berthe Porchet).—Fig. 15. Fibrovascular bundle of an old leaf collected Jan. 10, 1936, from an apricot tree grown in a nutrient solution containing 1 p.p.m. zinc.—Fig. 16. Transverse section of a fibrovascular bundle of a healthy peach leaf collected August, 1936, at San José.—Fig. 17. Transverse section of a fibrovascular bundle of an affected peach leaf collected August, 1936, at Delhi.

cot leaves collected July 19 at Milpitas. Vacuolization of the stromata and shrinkage were the initial steps in a process of disintegration which terminated in the disappearance of all chloroplasts from the cells.

The processes of lysis which are active during the summer seem to convert the cell contents into materials which cannot be detected microscopically. Although the plastids contained starch granules or oil droplets at an earlier stage, no traces of such substances could be seen, although the leaf material was fixed in solutions containing osmic and chromic acids.

The lipo-protein complex of the plastids seems to have been easily altered by the anisotropy incident to the little-leaf disease. The colloidal proteins, having been hydrated or hydrolyzed, were no longer stable with respect to the lipid substances. Thus one sees vacuolization of the plastid and segregation of oil droplets in the same plastids. In extreme cases (where illumination was strong) the irreversible changes in equilibrium led to degeneration of the plastid.

The cells adjacent to the dorsal epidermis were more affected than those in the median layers, and this was not always dependent on their proximity to the veins. It appears that the lytic processes mentioned proceed more rapidly in cells which are more strongly illuminated. Cells adjacent to endodermis showed the least disorganization of plastids.

It is highly important to note that the cytoplasm and nuclei of the leaf cells may resist for some time the lytic factors which attack the chloroplasts. Figure 11 shows the condition of a hypoplastic cell of the apricot leaf in which only the nucleus and one plastid remained.

It is important to note how the stability of the plastids is maintained by the application of a small amount of zinc to the tree. Although small oil droplets may retain their identity in the stroma, chlorophyll is produced and efficient formation of starch proceeds, while the plastid maintains its integrity as a synthesizing unit. The characteristic green color of the leaf appears as the zinc compounds reach the leaves. Without the application of zinc, the number and size of the oil droplets increase, eventually reaching a stage where the few small starch grains in the plastids may be masked by the surrounding oil drops.

The plastids of seedling apricots grown in the greenhouse at Berkeley in pots of Delhi soil suffered like those from the field. Leaves which were collected October 7 were mottled and dwarfed. Many of the palisade cells contained no recognizable plastids; other cells, especially those near the fibrovascular bundles, contained normal plastids with starch grains. Between these extremes, there were plastids in various stages of vacuolization and flattening, indicating that disruptive factors were present.

The occurrence of plastid injury in seedlings grown in soil from the affected district in the weaker light of the greenhouse must be emphasized, since it shows that the disappearance of plastids is primarily conditioned on factors having their origin in the soil and secondarily conditioned by light factors.

Peach.—Leaves of affected trees collected August 12 and fixed in Meves' solution contained plastids which were extremely vacuolated and contained oil drops (fig. 12). Some plastids were also flattened. Leaves in the same sample contained cells which showed lysis and incipient polarization of the cell structures, although there were large starch grains in the plastids. Apparently the processes of starch transformation had been inhibited. At the same time many palisade cells contained phenolic material, and mesophyll cells contained sterinoplasts.

More mature affected leaves collected on the same day and fixed in the same way showed evidence of less derangement of the plastids. Aside from incipient vacuolization, they appeared normal and were not filled with starch. Presumably these leaves had been less affected and had escaped the early abscission which had removed the more severely affected leaves.

Fresh sections of peach leaves from affected trees at Delhi examined August 7 confirmed what was observed in fixed material. Starch was generally present, although the older had less than the younger leaves.

Sunflower.—When sections of healthy and affected leaves are examined under the lower powers of magnifications, little difference in their organization may be seen, but when higher magnification is used, a profound difference in cell contents is seen, especially in the plastids.

The palisade cells of healthy leaves collected February 19 from plants in soil from Riverside contained plastids which absorbed dyes readily and contained an average of three starch grains each. In many cells the plastids completely lined the parietal walls.

The palisade cells of dwarfed, mottled leaves collected from plants of the same age grown in Delhi soil contained hyperchromatic, scattered plastids which were scantily supplied with starch grains. If starch was present, the grains were few and small. Their appearance suggested that some lytic process akin to gelatinization had been operating. The absence of organized structures in many of the mesophyll cells was also evidence of the action of lytic factors.

Squash.—The same general contrast in plastid development was found in the cells of leaves of a cucurbit grown in Delhi soil in the greenhouse (fig. 13). Typical little-leaf was prevalent in the plants, except in cases where zinc sulfate had been added to the soil in which they grew. The plants grew from seeds planted February 27, and the samples were collected April 8. The cells of healthy leaves contained many plastids which included starch grains. On the contrary, the cells of dwarfed, mottled leaves from soil minus zinc contained hypochromatic plastids in which starch grains were few and small. The cellular condition of the affected leaves (fig. 13, right) is a good illustration of the inhibition of plastid development in zinc-deficient plants.

Summarizing these observations, we see that derangements of plastid organization and function are first among the characteristics of the little-leaf dis-

ease and are to be considered responsible for many of the physiological perversions observed in affected plants. The lack of chlorophyll in portions of the leaves is so conspicuous that it has led to the designation of the disease in certain plants by terms such as "mottled-leaf," "yellows," and "white bud." In some plants—e.g., corn and Tung trees—there is a development of anthocyanin associated with the disease, but it does not occur in all plants.

The nature and extent of the plastid derangements are amenable to a study by cytological technic.

The lack of plastids is most conspicuous in the cells adjacent to the dorsal epidermis of the leaf and least in cells adjacent to the fibrovascular bundles.

Within the affected cells there is usually a definite agglutination of plastids accompanied by hyperchromatization of the plastid stroma and lack of starch. Injury to the plastid may not appear until after the plant has reached maturity, in which case the first evidence of the disease may be the hyperchromatization and vacuolization of the plastids. Oil droplets in the stroma of the plastids indicate that the colloidal structure has broken down. The ultimate stages in the derangement of affected leaves are reached when processes of lysis have destroyed the plastids and other organized bodies of the cell.

CELLULAR INCLUSIONS.—When a section of an affected leaf which was fixed in a solution containing chromates is examined, nothing is more conspicuous than the phenolic precipitates in the cells, especially if the leaf had reached maturity. Figure 14 shows this condition in the dwarfed leaf of a seedling apricot tree grown in a nutrient solution containing many accessory elements, but lacking zinc. The interpretation of this phenomenon is very difficult and has as yet been attended with only partial success. Part of this failure is no doubt due to ignorance of the chemical nature of tannin. Levi and Wilmer (1905) and Van Wisselingh (1910) produced evidence which strongly supports the idea that the tannin is a plastic material, useful for cell wall formation, though without it nuclear division may proceed without impediment.

Successive examinations of fresh and fixed apricot and peach leaves show that there is generally more phenolic material in affected than in healthy material. Nevertheless, the relation between phenolic compounds, such as tannins, to the little-leaf disease cannot be dogmatically stated, since they may be found to a certain extent in leaves of various ages.

Apricot.—Affected leaves collected April 20 at Delhi contained cells which appeared to be functioning efficiently, so far as the presence of starch grains was concerned, yet the vacuoles contained a small amount of phenolic material in the form of extremely fine granules. Palisade parenchyma cells adjoining the dorsal epidermis contained rather more phenolic material than the parenchyma cells in other parts of the leaf. Reference will be made later to the heavy concentrations found in the cells of the bundle sheath.

The parenchyma cells of healthy apricot leaves collected at San José contained no phenolic material. A comparison of leaves collected in July from both lots of trees showed that the amounts of phenolic materials was greater in the cells of affected leaves, yet there was relatively less in either than was found in April. Two explanations of this phenomenon are possible; either the badly affected leaves fell off before July or the leaves converted some of the phenolic compounds into something which was utilized in their metabolism, as van Wisselingh (1910) concluded.

With advancing age, the affected leaves of apricots at Delhi contained more phenolic material. On August 12, the palisade cells were moderately full of them. The abundance of these materials in other cells will be described later. Leaves of healthy apricot trees collected October 23 at San José, as well as mildly affected leaves collected the same day at Milpitas, had no cellular inclusions of phenolic or other abnormal materials.

Michel-Durand (1929) studied the tannin variations in branches of *Aesculus* detached from the tree at the initiation of the vegetation growth in the spring and found that the content of tannin in these branches was more constant than that of sugar. However, the tannin compounds appeared to participate in the development of the buds. After the development of the young organs had ceased, the residuary tannins produced by the chlorophyll synthesis migrated into the twig and were then converted into adsorbed tannins. When he placed excised twigs in darkness, the developing buds used the tannic substances more extensively. In the mature leaves, there was still less tannin and sugar. Apparently the greater losses were due to the prolonged utilization of the reserves in the period when photosynthesis was checked.

The leaf-cells of apricot seedlings grown in water cultures afforded material for some significant observations on the metabolic cycle involving tannins and related phenolic compounds. In certain cases zinc salts in the proportion of one or two parts per million had been added; in other cases no zinc was added. In the former case, the trees produced normal green leaves, in the latter they produced small, mottled leaves similar to those collected in the field (fig. 1). In June and July the amount of tannin in the cells was very small whether the trees had zinc supplied or not, but in November the parenchyma cells of both lots of trees were high in tannins and related substances, though in the leaves from the "zinc plus" cultures there was no macroscopic evidence of dwarfing or mottling. Tests for tannin were made on fresh sections as well as upon sections of fixed material.

The above observations were checked by investigations of fresh material obtained at Delhi on May 29. Fresh sections of affected leaves gave reactions for tannic material with caffeine, ammoniacal potassium iodide-iodine, and potassium bichromate. Neutral red penetrated the cells, demonstrated an enormous num-

ber of cellular inclusions by staining them dark brown, and stained fibrovascular elements red. On this date it was evident from these tests that phenolic materials had been formed in the leaf cells of affected apricot trees. Since no colors were developed by the Loew-Bokorny test for proteins, we may assume that disorganization of cell protoplasm had not started.

Thionine produced differential effects in the fresh sections. The cells of healthy apricot leaves absorb no thionine from aqueous solution, except the epidermal and vascular elements. The dye enters the vacuoles of the former and stains the walls of the latter violet color. The cells of affected apricot leaves, on the contrary, absorb thionine readily. Those which contain tannin change the color from violet to blue. Pure tannic acid acts the same way in vitro. By this useful dye, it is possible to demonstrate the presence of tannic material in fresh material.

Light intensity has a very important influence (as already stated) on the development of little-leaf symptoms. This factor also influences the production of tannin which was always more abundant in the palisade cells adjoining the dorsal epidermis. Confirmatory evidence on this question was obtained from a study of leaves collected October 7 from small affected apricot seedlings growing in Delhi soil in the greenhouse at Berkeley. The leaves showed every evidence of little-leaf conditions as shown by size and color of leaves, by the conversion of palisade parenchyma into rhomboidal parenchyma and by flattened plastids. There was, however, little tannic material in the leaf cells, though leaves collected from trees in the open, which received stronger illumination, were high in tannic compounds. The difference may be tentatively referred to differences in light intensity.

Peach.—Comparative studies on the cytophysiology of peach leaves also gave interesting results, but may be more briefly reported, since they revealed conditions similar in many ways to those reported for the apricot.

Affected trees at Delhi bore leaves which at an early stage had a few cells which contained phenolic precipitates, but the premature vacuolization of the cells was more striking (fig. 5). Fixed and stained sections of young leaves from affected trees showed small amounts of phenolic material on April 20, chiefly in the cells of the epidermis and of the endodermis.² Very young leaves collected from the apical region of the shoot contained a large number of fine globules in cells of both the upper and lower epidermis, but none elsewhere.

Various microchemical tests on fresh material from affected trees at Delhi, May 28, 1937, showed that starch was abundant, but no blue color was developed with the Loew-Bokorny test. There were lipoids demonstrated by nascent indo-phenol blue, but no

phenolic compounds could be demonstrated with ammoniacal potassium iodide-iodine.

Phenolic precipitates in parenchyma cells of affected peach leaves were not abundant during the summer, corresponding therefore to the response of apricot leaves. The tannin observed was principally in the endodermal cells. A certain amount of lysis was apparent, but a large proportion of the plastids were still present, and all contained starch grains (fig. 4).

Squash.—The differences between the cell structure of healthy and affected squash leaves shown in figure 13 were much like those of the peach leaf, but tannic material was not found.

Buckwheat.—The presence of phenolic materials in the leaves of affected buckwheat plants was a distinct feature of the little-leaf condition, since they were not found in leaves of healthy plants. The plants under investigation were grown in Sierra loam. The mottled, dwarfed plants had an average green weight of 8.6 grams; the healthy plants which had received zinc had an average green weight of 20.5 grams, indicating that the processes of growth were greatly restricted in the former case. The phenolic precipitates mentioned were found to be a general characteristic of palisade and endodermal cells of the dwarfed, chlorotic leaves.

If one might judge from the contour of the plastids and their starch content, the physiological processes in the buckwheat leaves were not seriously upset by the presence of the phenolic compounds.

Mustard.—The plants in pots to which no zinc sulfate was added were pale, and the leaves were dwarfed. Microscopical examination of the leaf cells showed that the leaves of the affected plants were composed of hypoplastic cells in various stages of disorganization, but without the presence of phenolic precipitates nor of lipid bodies, although the plastids were seriously disorganized.

Summarizing these observations, we may say that there is no evidence that the little-leaf disease is invariably characterized by the formation of phenolic compounds, although they were found in the most acutely affected plants. Species whose leaves were dwarfed by the disease contained more tannin. So far as the writer has investigated them in relation to little-leaf disease, he has found them present in apricot, avocado, buckwheat, orange, peach, and walnut leaves, and absent in corn, mustard, squash, sunflower, and tomato leaves. In the absence of more exact information, we can assume that tannins are elaborated variously from proteic or glucidic materials, notably in tissues in which the rate of metabolism is high—e.g., vegetative point, meristems, seeds in germination, buds, and leaves. It is hardly necessary to point out that extraordinary stimuli such as the puncture of an insect, which results in the formation of the oak gall, may result in a very abundant formation of tannin. The present work demonstrates that tannins may accumulate when cells are affected with physiological malnutrition.

²In the strict sense of the word, the sheath of cells surrounding the vascular bundles of the leaves described in this paper is not an endodermis. Consequently, the term "endodermis" is used only in the sense of a sheath whose cells usually contain starch.

CONDUCTING SYSTEM OF LEAVES.—The veins of affected leaves usually have green borders although the interveinous tissue may be pale yellow. No satisfactory postulate for the persistence of chlorophyll in this region has been given. One is tempted to say that the disease is due to a deficiency of some element, such as zinc, and that the supply is sufficient only for a narrow zone of cells on the borders of the conducting systems of the leaf. Analyses of healthy and affected peach leaves (Finch, 1936) bear out this idea, and Chandler, Hoagland, and Hibbard (1933) showed that affected peach leaves usually have a smaller zinc content.

The question naturally arises whether the functions of the conducting system of affected leaves are so impaired that they cannot convey the necessary quantities of essential elements to the centers of metabolism. Rand (1922) made an interesting observation on this question when he measured the diameters and areas of cross sections of large side veins of pecan leaflets. He reported that, although the total size of veins in the affected leaflets was approximately the same as that in healthy leaflets, the development of vascular tissue—i.e., the conducting elements—was appreciably smaller in affected leaflets.

The idea that mottling is due to an inadequate supply of some essential element is controverted by the results of experiments in which similar effects and patterns were produced by the use of poisons—e.g., leaves from orange trees which received beryllium or picric acid (Haas, 1932) and tobacco which received thallium (Spencer, 1937). In each of the cases cited, the veins had a green border, and the interveinous areas were yellow. Reed and Dufrenoy (1935b) demonstrated that zinc moved some distance from the veins to the cells of orange leaves having greater metabolic activity.

Reed and Dufrenoy also investigated the histology of the vascular elements of affected orange leaves, finding that they were less compact than those of healthy leaves. The condition of the living cells of the phloem received special attention, since they are important indicators of the physiological condition of the leaf. Evidence of necrosis was generally seen in the phloem of affected orange leaves, even at an early stage of mottling. The almost universal presence of phenolic bodies in the vacuoles of many cells and the swelling of the pectic material in the middle lamellae have been invariably associated with this type of physiological derangement in many trees and in some herbaceous plants. The importance of these alterations as criteria of physiological integrity has been abundantly established by repeated observations.

Stone fruits.—The onset of physiological derangements in the leaves of the apricot and peach is indicated by the formation of gums as well as phenolic compounds. One may distinguish the two substances from each other by the use of fresh sections, or by the use of Meves' solution for fixing the material, since the gums then stain bright red with acid fuchsin.

Maturity and senility of apricot leaves are marked by the presence of phenolic materials in the cells of the endodermis of healthy, as well as of affected apricot leaves (fig. 15). The difference is one of degree rather than kind, but can be established by careful observation. The condition of the vascular elements of affected leaves indicates that they were partially sealed in an envelope of gum-filled endodermal cells which could impede the movement of liquids through the channels.

The fibrovascular bundles of affected peach leaves showed no signs of abnormal conditions during the spring and early summer, although the leaves themselves were unmistakably mottled. The cells of the endodermis contained plastids which were usually ranged against the walls in contact with mesophyll cells, similar to what will later be shown in the cross section of corn leaves. Examination of fresh sections showed that the endodermal plastids of the peach leaf (either affected or healthy) contained starch.

Evidences of serious derangements in the fibrovascular bundles of affected peach leaves were subsequently found in material collected in August at Delhi, whereas healthy specimens collected at San José showed none of the derangements. If one compares figures 16 and 17, the nature of the derangement will be evident.

The endodermal cells of a healthy peach leaf (fig. 16) contained cytoplasm and healthy plastids, and the xylem and bast fibers were readily distinguished in their proper setting. The endodermal cells of an affected peach leaf (fig. 17) contained vacuolated plastids, unorganized material, and phenolic precipitates. In some cases the phenolic precipitates absorbed methyl green, but in most cases they had only the golden brown color due to their interactions with the chromates in the fixing solutions employed. The xylem elements were small in comparison with the size of the bundle; bast fibers were not found. The phloem parenchyma contained mitochondria.

Other plants.—The section of tomato leaf shown in figure 2 shows unmistakable cases of atrophied vascular system. The walls of the tracheae in squash and other leaves were brittle, as evidenced in cutting sections.

SUMMARY

The cells of leaves affected with the little-leaf disease show many changes which are associated with the deranged metabolism. A study of these units of biological architecture has advanced our understanding of the morphological and physiological characters of the affected plant and supplemented biochemical investigations.

The disease appears to promote cell growth rather than cell multiplication in the palisade parenchyma, and in the tomato there was atrophy of the mesophyll. There was also a lack of differentiation resulting in an essentially juvenile or, perhaps, xerophytic type of leaf structure.

The disease is characterized by destruction of chloroplasts or by a more or less complete inhibition of

their development. The cells receiving strongest illumination generally show the greatest plastid injury. Hypoplastic conditions are often associated with agglutination of the plastids in the polar region of the palisade cells; subsequently there is vacuolization and shrinkage of the plastid. Vacuolated or shrunken plastids seldom contain starch, except in the peach where, due to the lack of transformation into other carbohydrates, starch grains persist in the plastids of affected leaves. The factors producing derangement of the plastid equipment often seem to be localized, since adjacent cells may show none of the above-mentioned conditions.

Lytic factors associated with the disease frequently destroy most of the cell contents in more or less extensive areas.

The non-living inclusions of the cell serve as a valuable indicator of the amount, if not the nature, of derangement induced by the disease. The presence of phenolic substances in the cell vacuoles was a general character in certain leaves, like the apricot, but not in others. The leaf cells of corn, for example, were free from those substances, while in buckwheat the leaf cells contained phenolic materials only when showing marked symptoms of little-leaf. Observations on apricot and other leaves make it clear that phenolic substances occur in healthy as well as in affected leaves, leading to the conclusion that the differences may be one of degree rather than of kind. Differences in light intensity and, by implication, photosynthesis, appear to induce profound alterations in the tannins. With seasonal changes in the activity of growth, these substances tend to disappear from the cells of healthy leaves, but not from those of

affected leaves. There is no evidence that there was a toxic effect. It is more probable that the free phenolic substances may participate in the metabolism of these leaves, and that those which are adsorbed on colloidal substrates do not participate in cellular metabolism.

Sterinoplasts and gums in the cells of affected leaves are associated with other symptoms of hypoplasia.

On grounds which are as yet speculative, it seems that zinc salts may catalyze oxidation processes in the cells, and in their absence biochemical reactions may run the other way. Tannins may further impede processes of oxidation. The combined effects of these two factors may account for many of the derangements of the leaf cells.

The fibrovascular system of an affected leaf shows characteristic derangements. In peach, apricot, and other leaves, the endodermal cells are replete with phenolic substances, which, however, do not have any great tendency to diffuse, if one may judge from their reactions with salts of iron and chromium. In endodermal cells of mature or senescent peach and apricot leaves there may be plant gums.

Considering the microscopical structure of the entire leaf, we find that the elements of the fibrovascular system suffer less disorganization in the case of little-leaf than the cells of the palisade and mesophyll layers. The cause of the derangement of affected leaves is evidently associated with metabolism rather than with conduction.

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KEY TO ALL DRAWINGS

A, amyiferous plastids; B, non-amyiferous plastids containing vacuoles or oil globules; C, chloroplasts, reduced in size, starch usually present; D, stericoplast; EN₁, cell containing gum; EN₂, cell containing phenolic material; EN₃, cell containing cytoplasm and plastids; EP, dorsal epidermal cell; M, mitochondria; N, nucleus; P, palisade parenchyma; PH, phloem; PL, plastids; PR, pericycle; R, rhomboidal cell; T, phenolic precipitates; V, vacuole; X, xylem.

SEEDS AND SEEDLINGS OF THE TARO, *COLOCASIA ESCULENTA*¹

K. Kikuta, Leo D. Whitney, and G. K. Parris

TARO, *Colocasia esculenta* (L.) Schott, a monocotyledonous plant with net-veined leaves, has been propagated through the centuries by the asexual method, and seed production has been considered rare and of little significance. Young (1924) reports no setting of seeds by taro except in Hawaii, where an unidentified taro grower in 1913 produced several seedlings, and early in 1919 Gerrit P. Wilder of Honolulu was reported to have raised several hundred taro seedlings. An attempt to secure seeds by means of hand pollination in Florida was unsuccessful. MacCaughy and Emerson (1913) believe that fertile seeds rarely develop, and Willmott (1936) states that taro is believed to seed no longer.

One inflorescence of a taro plant of the "upland" variety *Elede Makoko* grown in the variety nursery of the Hawaii Agricultural Experiment Station of the University of Hawaii, has produced seeds naturally. Enlarged ovaries differentiated this inflorescence from those of other plants. The spathe was removed completely from the inflorescence, exposing the spadix, the staminate portion of which was already well dried. The enlarged ovaries varied from 3.0 to 5.0 mm. in diameter as contrasted with "sterile" ovaries which varied from 0.5 to 1.5 mm. in diameter. The plant was first observed December 5, 1936; on December 28 the inflorescence was removed, and 287 seeds were collected. The number of seeds in each ovary varied from 1 to 12.

The seed is 1.2-1.5 mm. in length, 0.7-1.0 mm. in diameter, hard, straw-yellow in color and conspicuously longitudinally ridged; ovate in shape resembling a miniature Japanese lantern, tapering toward the apical end with the apex beaked (fig. 1, A). There is a conspicuous hilum at the base, yellowish-green in contrast to the rest of the seed. The delicate, white, translucent funiculus usually remains attached after the seed matures.

Taro seeds were germinated between layers of moist filter paper, in soil, and in agar culture. In the first method, seeds were placed between layers of moist filter paper in Petri dishes at room temperature

(77°F.). Distilled water was added to keep the paper and seeds moist. A day later the seeds were swollen, and the longitudinal furrows began to fill out. In ten days the seed coat ruptured, and the radicle appeared from the anterior end (fig. 1, B). The radicle grew rapidly, as shown in figure 1, B-G. The typically bilobed and almost circular cotyledon next emerged (fig. 1, F-H). Chlorophyll developed almost immediately. The testa persisted on the cotyledon for a considerable length of time after germination (fig. 1, G). The first true leaf emerged as shown in figure 1, H. This leaf is typically almost circular, and the basal sinus or cleft is practically wanting (fig. 2, A); the depth of the sinus becomes more and more pronounced with each successive leaf (fig. 2, B-E). In the first true leaf the petiole is attached to the blade at its margin. With each successive leaf, however, the petiole tends to progress toward the center of the blade and, consequently, the leaf of a mature plant is more or less peltate or umbrella-shaped.

Seeds planted in soil emerged in 14 to 24 days, depending on the depth of planting. One seed in agar culture at room temperature germinated in 14 days. Seedlings grown in soil and agar developed in a manner comparable with those germinated on filter paper.

Due to its small size, taro seed is not thought to contain much stored food. The period of viability seems to be very short; seeds kept in an air-tight container failed to germinate after 30 days' storage.

The occurrence of this naturally developed seed raises the question as to whether the production of seed by taro is not more frequent than assumed and the event merely overlooked. It may now be possible to develop superior taro varieties by breeding if success attends attempts to produce seed artificially.

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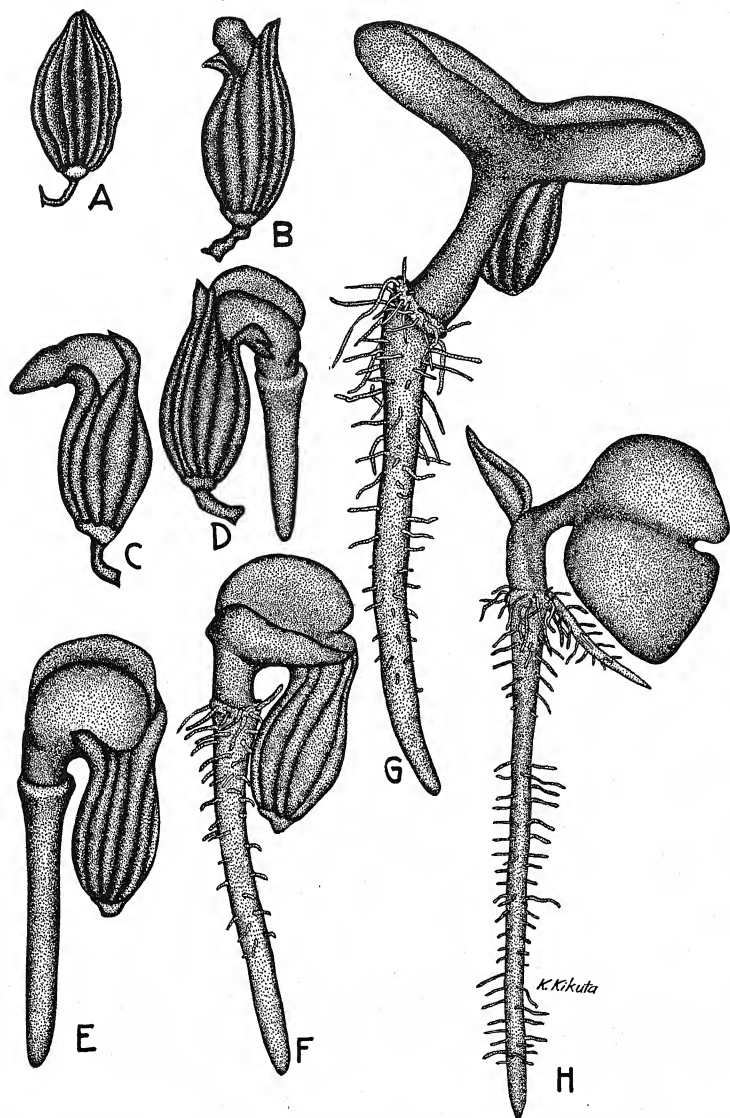


Fig. 1. Development of taro seedling: A, seed; B, 10 days; C, 11 days; D, 12 days; E, 13 days; F, 16 days; G, 18 days; H, 23 days. Drawings A-G under camera lucida $\times 26$; drawing H $\times 14$.

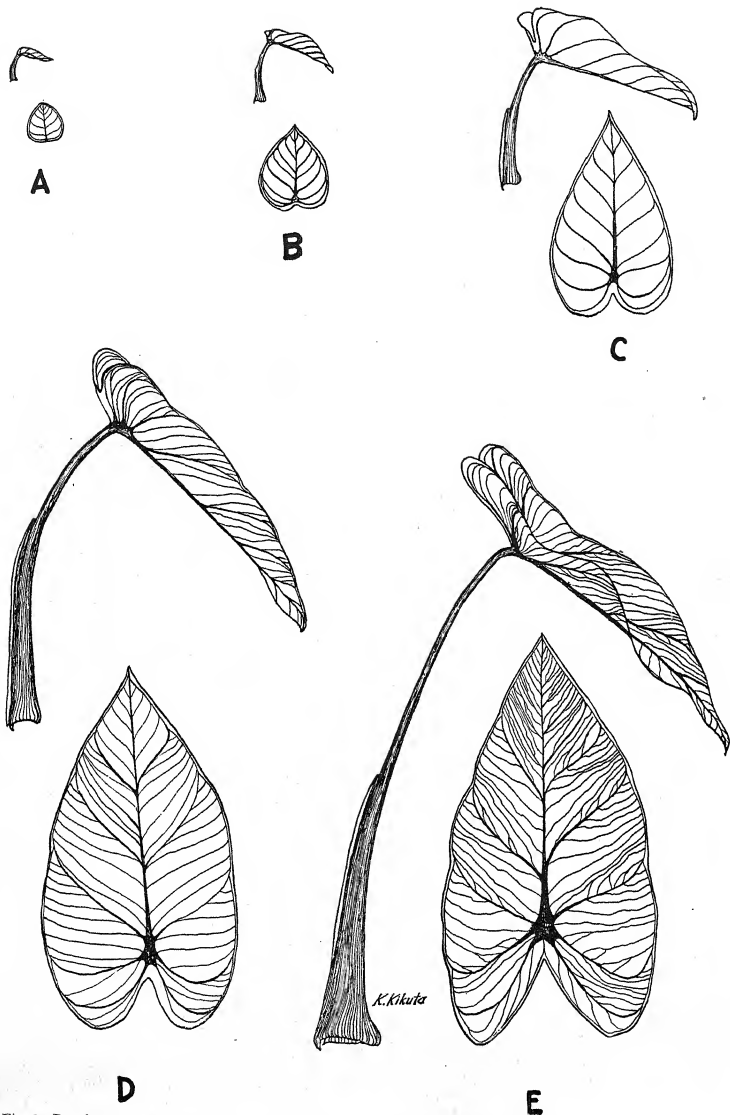


Fig. 2. Development of leaves of taro seedling: A, 1st leaf, 27 days; B, 3rd leaf, 55 days; C, 5th leaf, 80 days; D, 7th leaf, 93 days; E, 9th leaf, 103 days. All drawings $\times 17/20$.

CYTOLOGICAL CHARACTERISTICS ASSOCIATED WITH THE DIFFERENT GROWTH HABITS IN THE DICOTYLEDONS¹

G. Ledyard Stebbins, Jr.

IN REVIEWING the cytology of a number of different families of dicotyledonous angiosperms, the writer noted that although there were no characteristic differences between the chromosomes of the various larger systematic divisions of this subclass, there appeared to be, on the other hand, a definite set of characteristics associated with the different growth habits, particularly the woody plants as opposed to the herbs, and the annual or biennial as distinguished from the perennial species. In order to test this apparent phenomenon, he undertook a systematic review of the chromosomal characteristics of all dicotyledonous genera sufficiently recorded in the literature for this purpose. The results of this survey are recorded in the present paper.

MATERIAL AND METHODS.—Three chromosomal characteristics were selected as the easiest to treat in the manner contemplated: (1) the basic chromosome number of a genus, (2) the percentage of polyploid species within a genus, and (3) the absolute size of the chromosomes within the genus. The data for the first two characteristics were obtained chiefly from the lists of Tischler (1931, 1936), while additional information on certain genera was obtained from more recent partial lists and from a few treatments of particular genera. In general, genera included in the classification by basic number and by absolute size are those of which at least five species are known, but unless the known species were fairly uniform in character, they were not admitted to the lists unless a larger number of species was recorded. For classification as to percentage of polyploidy, only those genera were selected of which 30 per cent or more of the species are known cytologically, but an additional list was compiled including all the genera classified as to basic number.

The basic number was considered that which was found, either in itself or in multiples of it, in four-fifths or more of the species of the genus. If a second number occurred in a considerable proportion of the species, the genus was given one-half value under each number, while if there were three, it was given one-third value under each of them. If there were more than 3 numbers in the genus, only the 3 most common ones were recorded. Numbers near to multiples of the lowest basic number were considered to be derived by polyploidy—e.g., if a genus contained the gametic numbers 8 and 17, the latter was considered as a hypertetraploid based on the former. Only those basic haploid numbers actually found in the genus were recorded; the evidence from secondary pairing was disregarded as being not firmly enough established and not recorded in a sufficient number of genera to be of use in a comprehensive survey such

as this. A discussion of its possible significance to this problem is presented below.

To determine the relative frequency of polyploidy in genera of the different types, they were grouped into four classes: (1) those in which 0-25 per cent of the species had haploid numbers which were multiples or near multiples of the lowest one found in the genus; (2) those in which 25-50 per cent were polyploids; (3) those with 50-75 per cent, and (4) those with 75 per cent or more of polyploid species. Where polyploid series were recorded within the species, that species was recorded as one-half polyploid.

To determine the distribution of chromosome sizes, the chromosomes were grouped into four classes: (1) small (average length less than 2μ), (2) medium small ($2-5\mu$), (3) medium large ($5-9\mu$), (4) large (average length more than 9μ). The lengths were measured from all drawings of somatic plates which were clear enough and which had the scale of enlargement adequately given. The publications consulted for this purpose are too numerous to be cited here; for them the reader is referred to the lists of Tischler. Although errors must obviously have resulted from the fact that the use of different methods of fixation by different workers produced different degrees of contraction in the various chromosomal plates measured, the small number and large scope of the size classes recognized reduce the number of those errors so that, in the opinion of the writer, they are not numerous enough to affect results of a really decisive character. When the chromosomes of different species of a genus ranged over two of the size classes recognized, the genus was given one-half value under each size class.

DESCRIPTION OF RESULTS.—*Basic chromosome number.*—The following is a list of 151 herbaceous genera of dicotyledons classified as to basic number. In this list the strictly perennial genera are prefixed by the letter *P*, those exclusively annual or biennial by the letter *A*, while those containing both annual and perennial species are designated *a*. Genera given one-half value under a number are placed in parentheses, while those given one-third value are indicated as such.

n=4: a (<i>Leontodon</i>), a (<i>Hypochaeris</i>), a (<i>Crepis</i>).	11/2
n=5: a (<i>Verbena</i>), a <i>Picris</i> , a (<i>Hypochaeris</i>), a <i>Chondrilla</i> , a (<i>Crepis</i>)	3 1/2
n=6: A <i>Nigella</i> , P <i>Epimedium</i> , a (<i>Matthiola</i>), a (<i>Hesperis</i>), a (<i>Reseda</i>), a (<i>Lotus</i>), a (<i>Vicia</i>), a (<i>Trigonella</i>), a (<i>Viola</i> 1/3), a <i>Linaria</i> , a <i>Plantago</i> , P <i>Grindelia</i> , a <i>Hemizonia</i> 1/3, A (<i>Calycadenia</i>), a (<i>Leontodon</i>), a (<i>Tragopogon</i>), a (<i>Scorzonera</i>)	10 2/3
n=7: a (<i>Rumex</i>), P <i>Aquilegia</i> , a (<i>Ranunculus</i>), P <i>Thalictrum</i> , a <i>Papaver</i> , P <i>Thlaspi</i> , A <i>Sisymbrium</i> , a (<i>Matthiola</i>), a (<i>Hesperis</i>), a <i>Cochlearia</i> , a (<i>Crassula</i>), P	

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<i>Heuchera</i> , <i>P. Fragaria</i> , a (<i>Lotus</i>), a (<i>Vicia</i>), a (<i>Lathyrus</i>), a (<i>Trigonella</i>), <i>P. Ozalis</i> , a (<i>Impatiens</i> 1/3), a (<i>Sida</i>), a (<i>Oenothera</i> , a (<i>Godetia</i>) A <i>Cuscuta</i> , a <i>Phlox</i> , a <i>Lobostemon</i> , a (<i>Verbena</i>), a <i>Alectrolophus</i> (= <i>Rhinanthus</i>), <i>P. Valeriana</i> , a (<i>Valerianella</i>), a (<i>Cucumis</i>), a (<i>Hemizonia</i> 1/3), a (<i>Calycadenia</i>), a (<i>Lapia</i>), a (<i>Scorzonera</i>), a (<i>Ixeris</i>).....	25 1/6
n=8: a <i>Fagopyrum</i> , <i>P. Trollius</i> , a <i>Delphinium</i> , <i>P. Aconitum</i> , <i>P. Anemone</i> , a (<i>Ranunculus</i>), a <i>Adonis</i> , a <i>Lepidium</i> , a (<i>Biscutella</i>), <i>P. Barbarea</i> , a <i>Capsella</i> , a <i>Cardamine</i> , a <i>Draba</i> , a <i>Arabis</i> , a <i>Erysimum</i> , a <i>Alyssum</i> , a (<i>Crassula</i>), <i>P. Saxifraga</i> 1/3), a <i>Medicago</i> , a <i>Mcclintus</i> , a <i>Trifolium</i> , a <i>Crotalaria</i> , a <i>Astragalus</i> , a (<i>Linum</i> 1/3), a (<i>Impatiens</i> 1/3), a <i>Bupleurum</i> , <i>P. Eryngium</i> , a (<i>Gilia</i>), a <i>Echium</i> , a (<i>Cerinth</i>), a <i>Anchusa</i> , a <i>Galeopsis</i> , a <i>Antirrhinum</i> , a (<i>Valerianella</i>), a (<i>Knautia</i>), a (<i>Scabiosa</i>), a <i>Campanula</i> , a (<i>Lapia</i>), a <i>Madia</i> , a (<i>Ixeris</i>), <i>P. Taraxacum</i> , a (<i>Lactuca</i>), <i>P. (Prenanthes)</i>	35
n=9: a <i>Chenopodium</i> , <i>P. Mesembryanthemum</i> , a (<i>Biscutella</i>), a (<i>Brassica</i>), a <i>Raphanus</i> , a (<i>Lespedeza</i>), a (<i>Linum</i> 1/3), a (<i>Viola</i> 1/3), a (<i>Godetia</i>), <i>P. (Primula</i> 1/3), a <i>Polemonium</i> , a (<i>Gilia</i>), a <i>Nemophila</i> , a (<i>Cerinth</i>), a <i>Lamium</i> , <i>P. (Mentha)</i> , a <i>Nemesia</i> , a <i>Cephalaria</i> , a <i>Dipsacus</i> , a (<i>Scabiosa</i>), <i>P. Solidago</i> , <i>P. Aster</i> , a <i>Erigeron</i> , a (<i>Hemizonia</i> 1/3), a <i>Chrysanthemum</i> , a (<i>Centaurea</i> 1/3), <i>P. Hieracium</i> , a (<i>Lactuca</i>), <i>P. (Prenanthes)</i> , a (<i>Erodium</i>), a (<i>Geranium</i> 1/3).....	21 1/2
n=10: a (<i>Rumex</i>), a (<i>Polygonum</i>), a <i>Heliophila</i> , a (<i>Brassica</i>), a (<i>Reseda</i>), <i>P. Drosera</i> , a (<i>Sedum</i> 1/3), a (<i>Lespedeza</i>), a <i>Arachis</i> , a (<i>Impatiens</i> 1/3), a (<i>Viola</i> 1/3), a (<i>Knautia</i>), a (<i>Centaurea</i> 1/3), a (<i>Erodium</i>).....	7 5/6
n=11: a (<i>Polygonum</i>), <i>P. Rheum</i> , <i>P. (Saxifraga</i> 1/3), a <i>Phaseolus</i> , a (<i>Vigna</i>), <i>P. Opuntia</i> , a (<i>Convolvulus</i>), <i>P. Peucedanum</i> , <i>P. (Primula</i> 1/3), <i>P. (Asclepias)</i> , <i>P. Stapelia</i> , a <i>Phacelia</i> , a <i>Galium</i> , <i>P. Rubia</i> , a <i>Crucianella</i> , a <i>Asperula</i>	12 1/6
n=12: <i>P. (Dorstenia</i> 1/3), a <i>Silene</i> , a <i>Lychnis</i> , <i>P. Aethionema</i> , a (<i>Sedum</i> 1/3), a (<i>Vigna</i>), <i>P. (Primula</i> 1/3), <i>P. (Asclepias)</i> , a (<i>Convolvulus</i>), <i>P. (Mentha)</i> , a <i>Physalis</i> , a <i>Maurandia</i> , a (<i>Cucumis</i>), a <i>Coreopsis</i> , a (<i>Centaurea</i> 1/3).....	9 1/3
n=13: a (<i>Geranium</i> 1/3).....	0 1/3
n=14: <i>P. (Dorstenia</i> 1/3), <i>P. Nymphaea</i> , a (<i>Sedum</i> 1/3), <i>P. (Saxifraga</i> 1/3), a <i>Althea</i> , a (<i>Quamoclit</i>), <i>P. Digitalis</i> , <i>P. Antennaria</i> , a (<i>Geranium</i> 1/3).....	5 5/6
n=15: a <i>Dianthus</i> , <i>P. Crambe</i> , a (<i>Sempervivum</i>), a (<i>Linum</i> 1/3), a <i>Ipomoea</i> , a (<i>Quamoclit</i>).....	4 1/3
n=16: <i>P. (Dorstenia</i> 1/3), a (<i>Amaranthus</i>), a <i>Alchemilla</i> , <i>P. Ononis</i> , a <i>Verbascum</i> , <i>P. Dahlia</i>	4 5/6
n=17: a (<i>Amaranthus</i>), a (<i>Celsia</i>), a <i>Helianthus</i> , a <i>Cirsium</i>	3
n=18: a <i>Epilobium</i> , a (<i>Sempervivum</i>), <i>P. Symphytum</i>	2 1/2
n=20: a <i>Camelina</i> , a <i>Cucurbita</i>	2
n=21: a <i>Malva</i>	1
n=23-25: a (<i>Celsia</i>).....	1 1/2

A similar classification of the woody genera is as follows. The 51 whose numbers are definitely known are listed first, in italics, while an additional series, listed after the former in small Roman type, are included, based on such counts as are available, so as to make the number comparable to that of the herbaceous genera.

n=7: <i>Rubus</i> , <i>Rosa</i> ; <i>Hesperodes</i>	2 (3)
n=8: <i>Carpinus</i> , <i>Ribes</i> , <i>Spiraea</i> , <i>Prunus</i> , <i>Wistaria</i> ;	

<i>Ostrya</i> , <i>Theobroma</i> , <i>Myrica</i> , <i>Ostryopsis</i> , <i>Colutea</i> , <i>Punica</i> , <i>Aucuba</i> , <i>Clethra</i> , <i>Osmanthus</i>	5 (14)
n=9: <i>Physocarpus</i> , <i>Citrus</i> , <i>Daphne</i> , <i>Viburnum</i> , <i>Lonicera</i> , <i>Cistus</i> ; <i>Casuarina</i> , <i>Aeglopsis</i> , <i>Citropsis</i> , <i>Fortunella</i> , <i>Poincirus</i> , <i>Severinia</i> , <i>Carica</i> , <i>Incarvillea</i> , <i>Diervilla</i> , <i>Rhodotypos</i> , <i>Neviusia</i> , <i>Kerria</i> , <i>Grewia</i>	6 (19)
n=10: <i>Robinia</i> ; <i>Grevillea</i> , <i>Nandina</i>	1 (3)
n=11: <i>Coffea</i> ; <i>Fuchsia</i> , <i>Triplaris</i> , <i>Itea</i> , <i>Garrya</i> , <i>Calycanthus</i> , <i>Fendlera</i> , <i>Schizophragma</i> , <i>Cardiospermum</i> , <i>Myrtus</i> , <i>Alangium</i> , <i>Nerium</i> , <i>Gardenia</i> , <i>Pavetta</i> , <i>Psychotria</i> , <i>Phyllis</i>	1 (16)
n=12: <i>Quercus</i> , <i>Hamamelis</i> , <i>Vaccinium</i> , <i>Erica</i> , <i>Genista</i> ; <i>Protea</i> , <i>Cinnamomum</i> , <i>Persea</i> , <i>Garcinia</i> , <i>Myricaria</i> , <i>Fagus</i> , <i>Castanea</i> , <i>Pittosporum</i> , <i>Erythroxylon</i> , <i>Zizyphus</i> , <i>Leiothymum</i> , <i>Loiseleuria</i> , <i>Kalmia</i> , <i>Gaylussacia</i> , <i>Halesia</i> , <i>Corylopsis</i> , <i>Parrotiopsis</i> , <i>Fothergilla</i> , <i>Hovenia</i> , <i>Fatsia</i> , <i>Nothopanax</i>	5 (26)
n=13: <i>Ficus</i> , <i>Philadelphus</i> , <i>Deutzia</i> , <i>Acacia</i> , <i>Acer</i> , <i>Rhododendron</i> ; <i>Menispermum</i> , <i>Ledum</i> , <i>Arbutus</i> , <i>Coccolus</i> , <i>Mangifera</i>	6 (11)
n=14: <i>Corylus</i> , <i>Betula</i> , <i>Alnus</i> , <i>Morus</i> , <i>Ulmus</i> , <i>Berberis</i> , <i>Forsythia</i> ; <i>Cudrania</i> , <i>Arctocarpus</i> , <i>Cecropia</i> , <i>Illicium</i> , <i>Michelia</i> , <i>Celtis</i> , <i>Euptelea</i> , <i>Decumaria</i> , <i>Sarcococca</i> , <i>Eucalyptus</i>	7 (17)
n=15: <i>Rhus</i> , <i>Thea</i> , <i>Diospyros</i> , <i>Decasneuc</i> , <i>Liquidambar</i>	0 (5)
n=16: <i>Juglans</i> , <i>Carya</i> ; <i>Pterocarya</i> , <i>Jamesia</i> , <i>Eutonymus</i> , <i>Abelia</i> , <i>Kolkwitzia</i>	2 (7)
n=17: <i>Cydonia</i> , <i>Pyrus</i> , <i>Cotoneaster</i> , <i>Crataegus</i> , <i>Amygdalier</i> ; <i>Raphiophloeis</i> , <i>Eriobotrya</i> , <i>Hevea</i>	5 (8)
n=18: <i>Hydrangea</i> ; <i>Sambucus</i>	1 (2)
n=19: <i>Populus</i> , <i>Salix</i> , <i>Magnolia</i> , <i>Vitis</i> ; <i>Liriodendron</i> , <i>Drimys</i> , <i>Trochodendron</i> , <i>Tetracentron</i> , <i>Cercidiphyllum</i>	4 (9)
n=20: <i>Aesculus</i> , <i>Ampelopsis</i> ; <i>Davidia</i> , <i>Campsis</i>	2 (4)
n=21: <i>Platanus</i>	0 (1)
n=22: (<i>Syringa</i> 1/3).....	1/3 (1/3)
n=23: <i>Frazinus</i> , (<i>Syringa</i> 1/3), (<i>Cytisus</i>).....	1 5/6 (1 5/6)
n=24: (<i>Syringa</i> 1/3), (<i>Cytisus</i>); <i>Forestiera</i> , <i>Chionanthus</i>	0 5/6 (2 5/6)
n=41: <i>Tilia</i>	1 (1)

The genera containing both woody and herbaceous species may be classified as follows:

n=5: <i>Paeonia</i> , <i>Sphaeralcea</i>	2
n=6: (<i>Euphorbia</i> 1/3).....	1/3
n=7: <i>Potentilla</i> , <i>Lavatera</i> , <i>Abutilon</i> , <i>Pavonia</i> , (<i>Iberis</i>), (<i>Salvia</i> 1/3).....	4 5/6
n=8: <i>Pentstemon</i> , <i>Indigofera</i> , <i>Helianthemum</i> , (<i>Hypericum</i>), (<i>Salvia</i> 1/3).....	3 5/6
n=9: <i>Atriplex</i> , <i>Artemisia</i> , (<i>Pelargonium</i>), (<i>Hypericum</i>), (<i>Euphorbia</i> 1/3), (<i>Nicotiana</i> 1/3), <i>Cornus</i>	4 1/6
n=10: <i>Senecio</i> , (<i>Euphorbia</i> 1/3).....	1 1/3
n=11: (<i>Cornus</i>), (<i>Iberis</i>), (<i>Salvia</i> 1/3), (<i>Pelargonium</i>).....	1 5/6
n=12: <i>Solanum</i> , <i>Datura</i> , (<i>Nicotiana</i> 1/3).....	2 1/3
n=13: <i>Gossypium</i>	1
n=16: (<i>Nicotiana</i> 1/3).....	1/3

Figure 1 represents graphically the data from tables 1 and 2 as applied to woody vs. herbaceous genera. On this chart the herbaceous genera have a definite mode at the basic number 8, while the woody genera whose number is definitely established have no definite mode, but the great majority of them have 12 or more

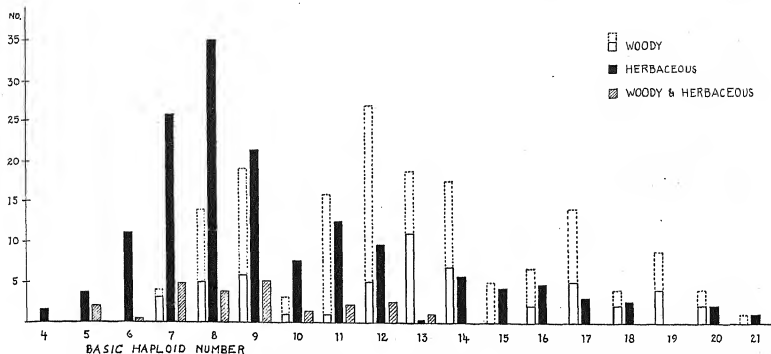


Fig. 1. Distribution of basic chromosome numbers in herbaceous and woody genera of dicotyledons. The height of the columns indicates the number of genera in each category.

as their basic number. If, however, those genera are added whose number is only tentatively known (shown on figure 1 by dotted lines), a conspicuous mode appears at the basic number $n=12$. In contrast to the herbaceous genera, therefore, those containing entirely woody species have, in general, higher basic chromosome numbers. The difference between the average basic numbers for the two types, 9 for the herbaceous and 14.2 (or 13.3 if the imperfectly characterized genera are added) for the woody group, about expresses the degree of difference between the two tendencies.

The small group of genera containing both woody and herbaceous species has nearly the same mode and average as the strictly herbaceous group. Within these genera no consistent difference could be found between the numbers of the woody species and those of the herbs.

The strictly annual genera and those containing some annual species have modes and averages similar to the herbaceous group as a whole, but the strictly perennial genera have no definite mode, and their average is slightly (though not significantly) higher. Hence the lower chromosome number of herbaceous as opposed to woody genera is partly correlated with the presence of annual species.

Another interesting fact in connection with the distribution of basic numbers is that the number is apparently more stable in the woody genera. Of the 51 woody genera definitely included in this tabulation, only 2, or 3.9 per cent, have more than one basic number; 96.1 per cent have only one. In the herbaceous genera, however, only 84 out of 151, or 55 per cent, have but one basic number; 42, or 28 per cent, have two, while 26, or 17 per cent, have three or more basic numbers. No significant difference in this respect can be found between the three groups of herbaceous genera—i.e., the genera with several

basic numbers are about equally distributed among those strictly perennial, those with some annual species, and those strictly annual. The genera with both woody and herbaceous species resemble the strictly herbaceous genera in that a large proportion of them possess several basic numbers.

PERCENTAGE OF POLYPLIIDY.—As mentioned above, the genera were for this purpose divided into two groups, group A containing all those of which 30 per cent or more of the species are known cytologically, and group B those which are less known. The value 30 per cent was chosen arbitrarily, in the absence of any simple statistical method of deciding which genera are well enough known for certain classification according to the method described above. The following is the classification of the herbaceous genera:

0-25 per cent polyploid. Group A: *P. Dorstenia*, a *Fagopyrum*, a *Nigella*, *P. Trollius*, *P. Epimedium*, a *Biscutella*, a *Thlaspi*, a *Raphanus*, *P. Barbarea*, a *Capsella*, a *Camelina*, a *Alyssum*, a *Matthiola*, a *Medicago*, a *Melilotus*, a *Vicia*, a *Linum*, *P. Primula*, a *Polemonium*, a *Phlox*, a *Nemophila*, a *Cerinth*, a *Celsia*, a *Antirrhinum*, a *Maurandia*, a *Nemesia*, a *Alectorolophus*, a *Crucianella*, a *Valerianella*, a *Knaulia*, a *Dipsacus*, a *Scabiosa*, a *Cucurbita*, a *Cucumis*, a *Calyceadonia*, a *Layia*, a *Crepis*, a *Lactuca*, *P. Prenanthes*.....39

Group B: a *Amaranthus*, a *Silene*, *P. Aquilegia*, a *Heliophila*, a *Arabis*, *P. Heuchera*, *P. Ononis*, a *Trigonella*, a *Crotalaria*, a *Lotus*, a *Lathyrus*, a *Phaseolus*, a *Vigna*, a *Lespedeza*, a *Impatiens*, a *Oenothera*, a *Epilobium*, a *Bupleurum*, *P. Peucedanum*, a *Phacelia*, *P. Asclepias*, a *Physalis*, a *Verbascum*, a *Linaria*, a *Cortopsis*, a *Leontodon*, a *Picris*, a *Hypochaeris*, a *Scorzonera*, a *Tragopogon*, a *Convolvulus*, a *Quamoclit*....32

25-50 per cent polyploid. Group A: a *Polygonum*, *P. Anemone*, a *Papaver*, a *Sisymbrium*, *P. Fragaria*, a *Malva*, *P. Symphytum*, a *Verbena*, a *Galeopsis*, a *Plantago*, a *Cephalaria*, *P. Dahlia*, a *Hemizonia*, a *Madia*, a *Cirsium*, a *Ixeris*, a *Chrysanthemum*.....17

Group B: a *Chenopodium*, a *Delphinium*, P *Ranunculus*, a *Lepidium*, a *Brassica*, a *Cardamine*, a *Reseda*, P *Drosera*, a *Crassula*, a *Sedum*, a *Sempervivum*, P *Saxifraga*, a *Trifolium*, a *Astragalus*, P *Oxalis*, a *Geranium*, a *Erodium*, A *Godetia*, P *Eryngium*, a *Anchusa*, a *Echium*, a *Lobostemon*, a *Lamium*, a *Asperula*, a *Helianthus*, a *Centaurea*, P *Aster*.....27
 75-100 per cent polyploid. Group A: a *Rumex*, P *Rheum*, a *Dianthus*, P *Aconitum*, P *Thalictrum*, a *Adonis*, P *Aethionema*, P *Crambe*, a *Viola*, P *Antennaria*, P *Taraxacum*, P *Grindelia*.....12
 Group B: P *Meibryanthemum*, a *Erysimum*, a *Sida*, P *Opuntia*, P *Stapelia*, A *Cuscuta*, a *Galium*, P *Rubia*, a *Erigeron*.....9
 75-100 per cent polyploid. Group A: P *Nymphaea*, a *Althea*, P *Mentha*, P *Digitalis*, a *Chondrilla*.....5
 Group B: a *Draba*, a *Hesperis*, a *Cochlearia*, a *Alchemilla*, P *Valeriana*, a *Campanula*, P *Hieracium*.....7

A similar classification for the woody group is as follows:

0-25 per cent polyploid. Group A: *Populus*, *Juglans*, *Corylus*, *Carpinus*, *Morus*, *Ulmus*, *Berberis*, *Philadelphus*, *Hydrangea*, *Physocarpus*, *Cydonia*, *Pyrus* (inc. *Malus*), *Wistaria*, *Robinia*, *Citrus*, *Aesculus*, *Vitis*, *Ampelopsis*, *Syringa*, *Foreythia*, *Diervilla*.....21
 Group B: *Quercus*, *Ficus*, *Ribes*, *Cytisus*, *Cistus*, *Daphne*, *Rhododendron*, *Erica*, *Prunus*, *Ligustrum*, *Viburnum*, *Lonicera*, *Coffea*.....13
 25-50 per cent polyploid. Group A: *Carya*, *Betula*, *Alnus*, *Spiraea*, *Amelanchier*, *Prunus*, *Tilia*.....7
 Group B: *Magnolia*, *Acacia*, *Acer*.....3
 50-75 per cent polyploid. Group A: *Desulzia*, *Rubus*, *Rosa*.....3
 Group B: *Salix*, *Crataegus*, *Vaccinium*.....3
 75 per cent + polyploid. Group A: *Coloneaster*.....1
 Group B: *Gemista*.....1

Table 1 shows a comparison of the two series. It will be noted that both in the better known genera (group A), and in the entire series (groups A+B), there are more woody genera with low percentages of polyploidy, and relatively more herbaceous genera with high ones. A statistical analysis, however, shows that this difference is not significant for group A.

The value $\chi^2=9.6$ was obtained by comparing this group in the two series. Since $n=7$ in this and the next three cases, P for this value of χ^2 is 0.2, and it therefore indicates a non-significant difference between the two series. For groups A+B, on the other hand, $\chi^2=28.4$, and the difference between them is therefore very significant ($P<0.01$). The difference, therefore, between the amount of polyploidy in the herbaceous genera and that in the woody ones is not significant according to our present knowledge, but may likely become so as more and more of the former genera are studied cytologically.

Within the herbaceous group, the relative frequency of polyploid series in the strictly annual genera (A), those strictly perennial (P), and those containing both annuals and perennials (a), is shown in table 1. In this table the much greater frequency of such series in the perennial genera is clearly shown, and for both series the difference is very significant ($\chi^2=28.5$ for group A, 39.4 for A+B; in both cases $n=11$, and

TABLE 1. Summary of the distribution of frequency of polyploidy. Figures outside of the parenthesis are for Group A (i.e., genera of which 80 per cent or more of the species are known cytologically). Those within the parenthesis are for Groups A+B.

	0-25%	25-50%	50-75%	75% +	Total
Herbaceous genera	39 (71) 53% (48%)	17 (44) 23% (30%)	12 (21) 17% (14%)	5 (12) 7% (8%)	73 (148)
Woody genera	21 (34) 66% (64%)	7 (10) 22% (10%)	3 (6) 8% (13%)	1 (2) 3% (4%)	32 (52)
Total	60 (105)	24 (54)	15 (27)	6 (14)	105 (200)
Annual (A)	12 (13) 85% (76%)	2 (3) 14% (18%)	0 (1) 0% (6%)	0	14 (17)
Perennial (P)	6 (11) 29% (29%)	4 (10) 19% (26%)	8 (12) 35% (32%)	3 (5) 14% (13%)	21 (38)
Both annual and perennial spp. (a) ..	21 (47) 55% (51%)	11 (31) 29% (32%)	4 (8) 11% (9%)	2 (7) 5% (8%)	38 (93)
Total	39 (71)	17 (44)	12 (21)	5 (12)	73 (148)

$P < 0.01$). If, therefore, only the strictly perennial herbaceous genera of group A are compared with the woody ones of the same group, the former show a very significantly higher frequency of polyploid series ($\chi^2 = 20.2$, $n = 7$) and even the total number of genera containing perennial species—i.e., the P and a genera together—show the same result ($\chi^2 = 16.4$ for group A, $n = 7$, $P = ca. 0.022$). Hence there is no doubt that the great tendency toward polyploidy characteristic of the angiosperms is manifest chiefly in perennial herbs and is less marked in woody plants as well as in annuals.

Chromosome size.—The classification of the herbaceous genera according to chromosome size is as follows:

Small ($< 2\mu$): *P (Dorstenia)*, a *Polygonum*, *P (Rheum)*, a *Rumex*, a *Chenopodium*, a *Amaranthus*, *P Mesembryanthemum*, a *Dianthus*, a *Silene*, *P Nymphaea*, *P Aquilegia*, *P (Thalictrum)*, a *Sisymbrium*, *P Barbarea*, a *Cardamine*, a *Erysimum*, a (*Hesperis* 1/3), a (*Alyssum*), a *Brassica*, *P Crambe*, *P Aethionema*, a *Lepidium*, a *Cochlearia*, a *Thlaspi*, a *Heliophila*, a *Raphanus*, a *Arabis*, a *Camelina*, a *Capsella*, a *Draba*, *P Drocera*, a *Sedum*, a (*Crassula*), *P Fragaria*, *P Saxifraga*, a (*Crotalaria*), a (*Trifolium*), a (*Astragalus*), a *Phacelia*, a *Lespedeza*, a (*Medicago*), *P (Oxalis)*, a (*Sida*), a (*Althea*), a (*Viola*), *P (Opuntia)*, a *Malva*, *P (Primula)*, a (*Cerinth*), a *Echium*, a (*Verbena*), a (*Galeopsis*), a *Verbascum*, a *Celsia*, a *Nemesia*, a *Maurandia*, a *Antirrhinum*, a (*Linaria*), a (*Plantago*), a *Cucumis*, a (*Scabiosa*), *P (Dahlia)*, a (*Madia*), a (*Izeris*).....50 5/6

Medium small (2–5 μ): *P (Dorstenia)*, *P (Rheum)*, a (*Rumex*), *P (Anemone)*, *P (Trollius)*, a (*Ranunculus*), a *Papaver*, *P (Thalictrum)*, a (*Hesperis* 1/3), a (*Matthiola*), a (*Alyssum*), a *Biscutella*, a (*Crassula*), a (*Crotalaria*), a (*Trifolium*), a (*Astragalus*), a (*Viola* 1/3), a (*Medicago*), *P (Oxalis)*, a (*Sida*), a (*Althea*), a (*Viola*), *P (Opuntia)*, a *Oenothera*, a *Godetia*, *P (Primula)*, a (*Cerinth*), a (*Verbena*), a (*Galeopsis*), a (*Linaria*), a (*Plantago*), a *Knautia*, a (*Dipsacus*), a (*Cephalaria*),

a (*Scabiosa*), *P (Dahlia)*, a *Coreopsis*, a *Hemizonia*, a *Layia*, a (*Madia*), a (*Calycadenia*), *P Antennaria*, a *Erigeron*, a *Chrysanthemum*, a (*Leontodon*), a (*Crepis*), a (*Izeris*), a *Centaurea*, a *Cirsium*, a (*Lactuca*), a *Chondrilla*, *P (Hieracium)*, *P Taraxacum*, a *Picris*.

32 2/3
Medium Large (5–9 μ): *P (Anemone)*, *P (Trollius)*, a (*Ranunculus*), *P Aconitum*, a *Delphinium*, a (*Hesperis* 1/3), a (*Matthiola*), a (*Viola* 1/3), a *Polemonium*, a *Anchusa*, a (*Calycadenia*), a (*Leontodon*), a (*Crepis*), a (*Lactuca*), *P Prenanthes*, *P (Hieracium)*.....10 1/6
Large ($> 9\mu$): a *Nigella*, a (*Viola* 1/3).....1 1/3

A similar classification of the woody genera follows:

Small ($< 2\mu$): *Salix*, *Populus*, *Juglans*, *Carya*, *Corylus*, *Ostrya*, *Magnolia*, *Morus*, *Ulmus*, *Ficus*, *Physocarpus*, *Rubus*, *Rosa*, *Prunus*, *Cydonia*, *Pyrus*, *Amelanchier*, *Crataegus*, *Robinia*, *Acacia*, (*Wistaria*), *Citrus*, *Acer*, *Tilia*, *Vitis*, *Ampelopsis*, *Frazinus*, *Syringa*, *Forstythia*.....28 1/2
Medium small (2–5 μ): *Staphylea*, *Quercus*, *Betula*, *Alnus*, *Ribes*, *Daphne*, (*Wistaria*).....6 1/2

For the genera containing both woody and herbaceous species, the classification is as follows:

Small ($< 2\mu$): *Potentilla*, *Pentstemon*, *Atriplex*, (*Iberis*), (*Solanum*), (*Salvia*), (*Senecio*), (*Lavatera*), (*Abutilon*), (*Pavonia*), (*Gossypium*).....7
Medium small (2–5 μ): *Sphaeralcea*, *Pelargonium*, (*Iberis*), (*Solanum*), *Datura*, *Nicotiana*, *Cornus*, (*Salvia*), (*Senecio*), (*Lavatera*), (*Abutilon*), (*Pavonia*), (*Gossypium*).....9
Large ($> 9\mu$): *Paonia*.....1

A summary of these lists is given in table 2. In this table the uniformly small size of the chromosomes of woody dicotyledons is strikingly shown. Moreover, although small chromosomes are common in both woody and herbaceous groups, medium-sized and large chromosomes occur with much greater frequency in the latter.

TABLE 2. Summary of the distribution of chromosome sizes.

	Small ($< 2\mu$)	Medium small (2–5 μ)	Medium large (5–9 μ)	Large ($> 9\mu$)
Herbaceous genera	50 5/6 53%	33 2/3 35%	10 1/6 11%	1 1/3 1%
Woody genera	28 1/2 81%	6 1/2 19%	0 0	0 0
Genera with herbaceous and woody species	7 41%	9 53%	0 0	1 6%
Annual (A)	6 1/2 52%	4 1/2 36%	0 1/2 4%	1 8%
Perennial (P)	12 1/2 57%	6 27%	3 1/2 16%	0 0
Both annual and perennial spp. (a)	31 5/6 52%	23 1/6 37%	6 1/6 10%	0 1/3 1%

When the different types of herbaceous genera, perennial, partly annual, and annual are compared, no significant difference can be found between the three groups. Apparently in the herbaceous dicotyledons as a whole, large chromosomes occur as frequently in annual as in perennial species. In some genera, such as *Crepis*, reduction in chromosome size is definitely associated with the reduction in the length of the life cycle of the plant and the appearance of the annual

habit (Babcock and Cameron, 1934). On the other hand, in *Iberis* (Manton, 1932), the annual species have definitely larger chromosomes than the related perennials, and in others, such as *Trifolium* (Wexelsen, 1928), although size differences exist, there is no clear correlation between them and growth habit. There is, apparently, some relationship between changes in chromosome size and the growth habit of herbs, but it is by no means a simple one.

Distribution of chromosome numbers and sizes in certain natural groups of Dicotyledons.—In order to see whether correlations similar to those described exist between chromosome number and size and the natural orders and families of dicotyledons, the distribution of these characteristics in five natural groups of dicotyledons, the Ranales, Cruciferae, Rosales, Tubiflorae, and Compositae, was tabulated (table 3). In regard to basic chromosome number, each group has the distribution of numbers expected from the relative number of woody and herbaceous species contained in it. The Ranales and Rosales, each of them containing a group of woody in addition to the herbaceous species, have modes at the numbers 12 and 14 as well as 8, while the other three, in which the species counted are predominantly herbaceous, have modes at 8 or 9.

cock and Cameron, 1934), phylogenetic reduction of chromosome size has been noted, while in other groups, such as the Gramineae (Aydulov, 1931) there is evidence for phylogenetic increase in size. Apparently these two tendencies occur with about equal frequency in the dicotyledons as a whole.

Discussion.—Significance of the differences in chromosome number.—The interpretation of the correlations obtained from this study must necessarily be somewhat tentative and speculative, at least until a larger number of genera is known cytologically. It must be remembered that this study is based very largely on genera of temperate and boreal regions. A study of the much larger number of genera, particularly in the woody group, that are confined to the tropics may yield entirely different results, although such data as have been obtained on them to date do

TABLE 3. *Distribution of basic numbers and size classes by families and orders.*

No.	Ranales	Cruciferae	Rosales	Tubiflorae	Compositae
4					11/2
5	1			01/2	3
6	2	1	11/2	21/3	35/6
7	21/2	41/2		41/2	21/3
8	51/2	81/2	125/6	41/3	4
9	0	2	41/2	51/6	81/6
10	1	11/2	25/6	0	11/3
11	1	01/2	45/6	1	0
12	2	1	61/2	25/6	11/3
13	2	0	3	0	0
14	4	0	12/3	1	1
15	1	1	11/2	0	0
16	0	0	2	1	1
17	0	0	6	01/2	2
18	0	0	11/2	1	0
19	6	0	0	0	0
20		1			
Size					
<2 μ	4 (33%)	17 1/3 (83%)	21 (81%)	10 1/2 (58%)	2 (9%)
2-5 μ	2 (17%)	25/6 (13%)	41/3 (17%)	51/2 (31%)	15 1/2 (74%)
5-9 μ	4 (33%)	5/6 (4%)	1/3 (1%)	2 (11%)	31/2 (17%)
>9 μ	2 (17%)		1/3 (1%)		

In regard to chromosome size, there are definite differences among the different groups. The Ranales and Compositae have definitely larger and the Cruciferae and Rosales definitely smaller chromosomes than the average for the five groups. These differences, however, are clearly not correlated with the position of the groups in any of the systems of phylogeny. The Ranales are generally regarded as primitive and the Compositae as advanced, while the groups with small chromosomes, the Cruciferae and Rosales, occupy an intermediate position. Hence chromosome size is an attribute of certain families and orders, but has no direct connection with the phylogeny of the angiosperms in the larger sense. In particular genera, such as *Muscari* (Delaunay, 1926) and *Crepis* (Bab-

not indicate this. Nevertheless, a consideration of the most plausible hypotheses for the explanation of these phenomena should be of value to future studies along this line.

In regard to basic number, the most obvious hypothesis is that the relatively high numbers of the woody dicotyledons are derived by polyploidy from smaller ones. There are two possibilities in this connection: either the woody genera are derived by polyploidy from the herbaceous ones, or they are so derived from other woody genera, either tropical types not yet studied cytologically, or extinct types.

The first possibility is not borne out by a comparison between woody and herbaceous species within the same genus or family. In none of the genera

containing both woody and herbaceous species are the woody species predominantly polyploid, and in many of them, such as *Paeonia*, *Potentilla*, *Pentstemon*, and *Nicotiana*, the polyploid species are all or nearly all herbaceous, while the woody species are diploid.

When different genera of the same family are compared, a similar discrepancy is evident. Although the herbaceous genera of the Rosaceae have on the average lower basic numbers than the woody ones, these numbers are not in a multiple ratio to each other. The predominant basic number among the herbaceous genera (e.g., *Potentilla*, *Fragaria*, and *Geum*) is 7, while among the woody genera, excluding the Pomoidae, 8 and 9 are the most frequent numbers. In the Leguminosae, 8 is the most common basic number among the herbaceous genera, while 9, 10, 12, and 13 are recorded for such woody genera as are known cytologically. The numbers 7 and 6, although they occur in the family, are found chiefly in such specialized types as *Vicia* and *Lathyrus*, which are better explained as having been derived from other, more primitive, herbaceous types than as being ancestral to such woody genera as *Robinia* and *Acacia*. The hypothesis that the woody genera have been derived by polyploidy from the herbaceous ones, therefore, does not agree with the evidence from the taxonomic and phylogenetic relationships of these genera.

For the hypothesis that the woody genera considered in this study are derived by polyploidy from other woody types there is considerably more support. In the first place, secondary pairing is frequent among these genera, the Pomoidae forming a classic example of this phenomenon (Darlington and Moffett, 1930). Secondary pairing as a criterion of polyploidy has been supported by Darlington (1937, p. 236-243), Catchside (1937), and others, but is doubted by Heilborn (1936). In the opinion of the present writer, the evidence in favor of the significance of secondary pairing is more significant than that against it, and in some groups, such as *Dahlia* and the Pomoidae, this significance is well established. Nevertheless, secondary pairing may not be considered proof of polyploidy in the absence of other evidence for it.

Further evidence for the polyploid nature of some of the woody genera here studied is the fact that their numbers are either multiples of those found in other related woody genera or are so high as to suggest a priori a polyploid origin. The Pomoidae are undoubtedly derived by polyploidy from other woody Rosaceous genera (Darlington and Moffett, 1930; Sax, 1932), while the Juglandaceae, most genera of Oleaceae, the genera *Tilia* and *Cytisus*, as well as other groups, are almost certainly of polyploid origin.

Difficulty arises, however, in interpreting as polyploid in origin the three numbers higher than 9 found most frequently among the woody genera—namely, 11, 12, and 14. If these numbers were derived directly by polyploidy, the genera possessing them must have had ancestors with the basic numbers 5, 6, or 7. The first two numbers have not been definitely established for any strictly woody genera, while the number 7 is

established for this group only in the relatively specialized and probably modern genera *Rosa* and *Rubus*. Among the genera containing some woody species and with numbers of 5 to 7, only *Paeonia* may be considered primitive; *Potentilla*, *Euphorbia*, *Salvia*, and the various genera of the Malvaceae are certainly specialized, derived types. Therefore, the hypothesis of a polyploid origin for the numbers 11-14 in the woody genera depends on the assumption of the existence of 5-7 chromosome types either among tropical groups as yet not studied or among extinct types. Although the latter assumption can never be either proved or disproved, the presence or absence of genera with low numbers in relatively primitive tropical families will provide a valuable criterion of the validity of this hypothesis. On the basis of the present evidence, however, the assumption of a direct polyploid origin for the numbers 11-14 in the woody dicotyledons is improbable. The indirect origin of these numbers, however, through doubling of an original set of 8 or 9 with consequent loss of 2 or more pairs of chromosomes is worth considering. Concerning this hypothesis also a study of the woody genera of the tropics will provide critical evidence. If the more primitive of these genera have either the basic numbers 16-18 or 8 and 9, while the numbers 11-14 are found in those genera definitely derived or those most nearly related to temperate groups, the hypothesis of indirect polyploidy for the origin of these genera will receive strong support.

However, in the absence of definite evidence for the polyploid origin of these woody genera, either directly or indirectly, another hypothesis concerning their origin is worth considering. This was suggested by the presence of the basic numbers 11 and 12 in nearly all woody gymnosperms and of numbers as high or higher in most living pteridophytes (cf. Tischler, 1931, 1936). The suggestion therefore arises that the gymnospermous and pteridophyte ancestors of the woody angiosperms had the basic numbers 12-14 and that the woody dicotyledons with these numbers have been derived from them with little or no change in basic number. The herbaceous types with lower numbers, which according to anatomical evidence are in general derived from woody types, have come either from pre-angiospermous herbaceous types with low numbers or from woody angiosperms by a gradual reduction in the basic number. The latter is definitely the most likely origin for the herbaceous Rosaceae, Leguminosae, and Urticales, which have close relatives among woody genera with higher numbers. Evidence for the gradual reduction of the basic number has been obtained in the Polygonaceae (Jaretsky, 1928), Cruciferae (Manton, 1932), Gramineae (Ardulov, 1931), the genus *Crepis* (Babeok and Cameron, 1934), and other groups.

This second hypothesis is in good accord with the second attribute of woody genera—i.e., the relative stability of their chromosome numbers. One would expect that this present stability has always been true of them, rather than that they have gone through a

complicated series of changes in basic number in the past and have only recently become stable. On the other hand, the present tendency for variation in basic number among the partly or wholly herbaceous genera suggests that in most of these the primitive basic number has been altered.

A possible explanation for the cytological stability of the woody genera as compared with the herbs is the relative constancy of the growth rhythm and habit of the former. For instance, the different types of growth habit, as determined by the life forms of Raunkiaer (1934), are much more numerous in herbaceous than in woody genera. This suggests the hypothesis that gross changes in the number, absolute size, and perhaps also the structure of the chromosomes are usually accompanied by changes in the growth habit of the plant and that in this correlation is to be sought the greater cytological instability of the herbaceous genera.

Significance of the differences in the percentage of polyploidy.—In connection with polyploidy two facts must be explained: first, the relative infrequency of polyploidy in annual as contrasted with perennial herbs, and second, the smaller percentage of polyploid series found in woody genera. For the first fact one explanation is undoubtedly valid. Amphidiploidy—i.e., chromosome doubling after hybridization—is undoubtedly more easily accomplished in perennial species. If a sterile hybrid is annual, it must form in a single season either a tetraploid shoot or unreduced gametes which function. On the other hand, a sterile perennial, if it is vigorous, may live indefinitely and even propagate itself vegetatively to form a large clone, as is illustrated by the classic examples of *Primula kewensis* and *Spartina Townsendii*, until finally the fortuitous combination of circumstances appears to produce the fertile amphidiploid. This provides ample evidence for the high frequency of polyploid species in strictly perennial genera.

There is evidence, however, that the infrequency of polyploidy in some annual groups is not due entirely to the poor chance for their occurrence. Between two annual species of *Crepis* (Hollingshead, 1930), an amphidiploid when obtained experimentally was not only sterile but small and weak in growth. This indicates the presence of physiological barriers to the building up of polyploid series within this group of *Crepis* species, although in other species of the same genus, chiefly the perennial American species, such series are highly developed.

Another type of barrier to the building up of polyploid series in annuals is suggested by the observation of Dermen (1936) that polyploidy "accelerates vegetative growth and decreases normal sexual development." In annual species the reproductive phase of growth must be highly developed—i.e., the flowers and fruits must form a relatively high percentage of the bulk of the plant—and any disturbance of the growth pattern tending to hinder this phase would be disadvantageous to the plant as a whole.

In an extensive review of this subject, Muntzing (1936) has clearly demonstrated that the average chromosome number of perennial species "is not less than 59% higher than that of the annual species" (i.e., p. 352). He concludes from this fact that "a large number of perennial species must have originated from annual types with lower chromosome numbers" (p. 356). The present study, however, shows that this conclusion is not justified, because of the high frequency of polyploids in genera of which the *diploid species as well as the polyploids are perennial*. This probably holds also for the different subdivisions of many genera in which both annual and perennial species occur. In most of the genera in Muntzing's list which show a striking difference between the average chromosome number of the perennials and that of the annuals; i.e., *Polygonum*, *Galium*, *Veronica*, *Erysimum*, *Medicago*, *Trifolium*, *Festuca*, and *Bromus*, the annual species are usually in different, often more highly specialized sections than the perennials, while in others, i.e., *Helianthus*, *Senecio*, *Papaver*, *Mercurialis*, *Urtica*, *Chenopodium*, *Alopecurus*, and *Anthoxanthum*, the number of species included by him is too small to be of great significance. There is, of course, definite evidence that polyploidy may sometimes transform an annual into a biennial or perennial, but there is an equal or greater number of cases in which this is not true, as is evident from the presence of 50 polyploid annuals on Muntzing's list. The evidence presented by the writer indicates that the vast majority of polyploid perennials are descended from perennial diploid ancestors, and therefore that Muntzing's data support the conclusion of the present author.

For the other difference—i.e., between perennial herbs and woody plants—a different explanation is obviously necessary. The following possible ones have occurred to the writer:

Polyploidy disturbs the size relationships of the cells so that wood fibers cannot be formed satisfactorily by the activity of the cambium. Favoring this hypothesis is the fact that all of the eight genera with 50 per cent or more of polyploidy consist chiefly of shrubs rather than trees. Against it are the presence of a high basic number in such genera of trees as *Tilia* and *Frazinus*. In these, the basic number itself must be of polyploid origin, even though polyploid series within the genus are not very strongly developed.

The cytological stability of woody plants makes the occurrence both of somatic doubling and of unreduced gametes less common in them. For the latter fact there seems to be good evidence in the regular meiotic behavior of the chromosomes in hybrids between species of such woody genera as *Rhododendron* (Sax, 1930), *Platanus*, and *Campsis* (Sax, 1933). In these genera amphidiploidy following hybridization would not be expected to occur. This explanation seems to be the most likely one at present. It is strengthened by the fact that a large proportion of the exceptional woody genera with higher percentages of polyploidy

belong to the Rosaceae, a family of which the genera, both woody and herbaceous, are noted for the frequency of natural hybridization and polyploidy within them as well as for the morphological and genetical instability of their species.

Significance of the differences in chromosome size.—

In regard to the absolute size of the chromosomes only one fact needs to be explained—i.e., the uniformly small size of the chromosomes of the woody genera. A logical explanation of this fact presents itself in the cytological stability of woody genera. We might assume that the presence of uniform, small chromosomes was characteristic of the ancestors of the angiosperms and that the woody genera have retained this condition as well as the primitive chromosome number. Against this hypothesis is the relatively large size of the chromosomes in all living gymnosperms and in most pteridophytes. The fact that such different groups of gymnosperms as the conifers, cycads, and Gnetales have chromosomes similar to each other in their large size and general morphology makes the supposition very likely that similar large chromosomes were possessed by the gymnospermous ancestors of the angiosperms.

A second hypothesis to explain this small size of the chromosomes of woody dicotyledons is that of Darlington (1937, p. 84)—i.e., that the nucleus and cell must be small if cambial activity is to take place. The obvious objection to this hypothesis, however, is the well developed cambial activity of the gymnosperms, in which the chromosomes are not only large but relatively numerous, so that the chromosomal volume of the nucleus is greater than that of all but a few of the herbaceous dicotyledons.

To be sure, the dimensions and volumes of both the mature cells and the cambial initials of the vascular tissue are quite different in angiosperms, and some incompatibility between large cell volume and cambial activity may exist in them which is not found in gymnosperms. However, any modification of Darlington's hypothesis along these lines would have to be based on a careful comparative study of chromosome size and the volume of cambial initials throughout the dicotyledons.

GENERAL CONCLUSIONS.—Perhaps the most significant conclusion to be made from this study is that there is a definite correlation between habit of growth and the cytological characteristics of the angiosperms and that this correlation, furthermore, is stronger than any that exists between cytological differentiation and the differentiation of the higher plants into families and orders. In other words, the evolution within the same genus or family of new species with different habits of growth has in general been accompanied by more visible changes in the chromosomes than has the evolution of new families or orders. A logical continuation of this type of study would be to determine to what extent such differences as those between the temperature, moisture, and soil requirements of different plants are correlated with cytological differences.

SUMMARY

Two hundred and five genera of woody dicotyledons, 151 of herbaceous dicotyledons, and 22 containing both woody and herbaceous species were compared as to basic chromosome number, 52 and 148 genera of the woody and the herbaceous groups, respectively, as to percentage of polyploidy, and 35, 96, and 17 of the three groups, respectively, as to absolute chromosome size, using data obtained from the published chromosome lists and from numerous publications on particular groups.

The basic numbers are significantly higher in the woody group, and there is much less variation in basic number within the same genus. In these respects the genera containing both woody and herbaceous species resemble the strictly herbaceous ones.

Polyploid series are most frequent in strictly perennial herbaceous genera and are less frequent in both the strictly annual and the woody groups.

The great majority of woody genera have small chromosomes, while the larger size classes are relatively frequent among herbaceous genera and among those containing both woody and herbaceous species.

The relatively high basic number of the woody genera is explained partly by the polyploid origin of many of them. Those with the basic numbers 11, 12 and 14, however, may inherit these numbers from their gymnospermous ancestors, while the numbers 7, 8, and 9 most characteristic of herbaceous dicotyledons may be derived from the former numbers by reduction.

The cytological stability of woody genera as made evident by relative uniformity in chromosome size and number and a relatively high degree of chromosome compatibility in interspecific hybrids is correlated with their relative stability in habit.

The relatively low percentage of polyploid species among annuals as contrasted with perennial herbs is due largely to the relative difficulty of the formation of amphidiploid hybrid derivatives in the former group. The effect of polyploidy in disturbing the growth pattern is, however, another significant factor.

The infrequency of polyploidy among woody genera is probably another manifestation of their cytological stability.

The hypothesis of Darlington concerning the relationship between chromosome size and cambial activity in woody plants is incompatible with the situation found in the gymnosperms, but a modification of it applying only to angiosperms is possible.

Gross changes in the chromosomes have been more significant in producing new types of growth habit than in differentiating new families and orders.

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CULTURE AND INOCULATION STUDIES ON RACES OF THE LOOSE AND COVERED SMUTS OF OATS¹

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RECENT STUDIES on the cereal smuts may be grouped into four main lines of investigation: (1) specialized races, (2) culture behavior, (3) heterothallism, and (4) hybridization. The specialized races are distinguished from one another by their physiologic behavior on selected, differential host varieties—neither the pathogen nor the infected host showing distinctive morphological characteristics for a given race. A knowledge of the number, distribution, and infective capacities of these races is of primary importance in the production of immune or resistant cereal varieties, and race identification by other methods than host infection would save both time and labor in experimental procedure. Consequently, numerous observations have been made for characteristics which might differentiate races of the various smuts when grown on artificial culture media.

Basic to the problem of heterothallism is an understanding of the nuclear condition throughout the life cycle of the smuts. The oat smuts produce multinucleated mycelia in the host which break up into numerous binucleated cells upon the initiation of chlamydospore production. The immature chlamydo-

spore is binucleated but, by fusion of these haploid nuclei, becomes uninucleated. Germination of the chlamydospore is associated with two successive divisions of the nucleus, four haploid nuclei being present in the promycelium. Septa appear between the nuclei, and from each promycelial segment a uninucleate sporidium is developed. The heterothallic nature of the sporidia is supported by considerable evidence and has provided means for hybridizing smut species and races. Consequently, unlimited possibilities are provided for the origin and production of new races. Fusion between certain sporidia, but not their nuclei, restores the binucleate and multinucleate condition.

Culture studies have been reported for both the loose smut (*Ustilago Avenae* (Pers.) Jens.) and covered smut (*U. levis* (K. & S.) Magn.) of oats. Rodenhiser (1928) grew multichlamydospore cultures of eighteen collections of loose smut and five of covered smut; he concluded that their cultural characteristics were adequate for differentiating races. Greater differences were observed in some cases between the cultured races of a species than those noted between different species. Kingsley (1933) found single chlamydospore cultures unreliable for distinguishing different races, since variations of cultural characteristics within the same physiologic form often

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were as great as between the different forms. She believed this to be the result of different genetic variations between sporidia derived from the germinated chlamydospores.

Permanence and segregation of cultural characteristics of *Ustilago levis* were studied by Dickinson (1928), who cultured the primary sporidia isolated from several chlamydospores and found them to be distinct from one another. Segregation for different cultural characteristics occurred on a 2:2, 3:1, or 4:0 basis, and the permanence of these characteristics was established. Isolation of successively formed sporidia from a given promycelial segment indicated that their cultural characteristics were identical with those of the primary sporidium. Segregation occurred in the first or second reduction divisions and appeared to be independent for the different character factors. The same author, in 1931, presented the results of studies on the effect of external conditions on segregation of cultural characters. Hüttig (1931) reported the effect of different temperatures on segregation of characters in monosporidial isolations of *U. Avenae*. Holton (1932), using both oat smuts, cultured successive single sporidia isolated from the same promycelial segments. He concluded that segregation of cultural characters might occur in the first or second, or even later, divisions of the nucleus, while "sex" segregated only in the first and second divisions. Culture variants were observed in these monosporidial lines and were believed to be the result of extended or delayed reduction for cultural characters, rather than mutation. Nicolaisen (1934) reported marked growth differences among various monosporidial cultures which he considered indicated that a given smut collection included a population of morphologically distinct, mostly heterozygotic, biotypes. Leitzke (1937) cultured single sporidia from certain loose smut collections and from their growth behavior he concluded that his collections contained a mixture of physiologically different forms in which the greater number of their spores were heterozygotic.

Heterothallism was first demonstrated in the smuts by Kniep (1919), who noted that fusion occurred between certain sporidia of *Ustilago violacea* (Pers.) Fuckel and that these sporidia were produced in equal numbers. Bauch (1922) worked with the same smut species and observed that mixtures of monosporidial cultures of opposite "sex" gave different types of growth compared with those of the individual cultures or combinations of cultures of the same "sex." This phenomenon has been studied in the oat smuts by Dickinson (1927a, 1927b, 1928, 1931), Hanna and Popp (1930, 1931), Holton (1931, 1932, 1936a), Nicolaisen (1934), and Popp and Hanna (1935).

Hybridization has been reported in the oat smuts. Dickinson (1927a, 1927b) claimed that *Ustilago levis* hybridized with *U. hordei*, but he did not recover chlamydospores from mature inoculated plants. Hanna and Popp (1930, 1931) obtained hybrids between the two oat smuts by pairing monosporidial culture

lines. Holton (1931, 1932, 1933) made interspecific crosses and studied the inheritance of chlamydospore characteristics in the hybrids. Kingsley (1933) and Nieves (1934) observed smut types which they believed resulted from natural hybridization. Nicolaisen (1934) suggested that new races of loose smut could arise through the crossing of known ones. Popp and Hanna (1935) studied the inheritance of spore characteristics of hybrids by the back-cross method. Holton (1936a, 1936b), also, investigated the inheritance of chlamydospore characters and described certain new strains resulting from hybridization of the oat smuts.

Many other investigators have also reported on cultures, heterothallism, and hybridization in *Ustilago Zeae* (Beckm.) Ung., *U. Tritici* (Pers.) Rostr., *U. hordei* (Pers.) K. & S., *U. nuda* (Jens.) K. & S., *U. violacea* (Pers.) Fuckel, *Sphacelotheca Sorghi* (Link) Clint., *S. cruenta* (Kühn) Potter, *Sorosporium Reilianum* (Kühn) McAlp., *Tilletia laevis* Kühn, *T. Tritici* (Bjerk.) Wint., and other species.

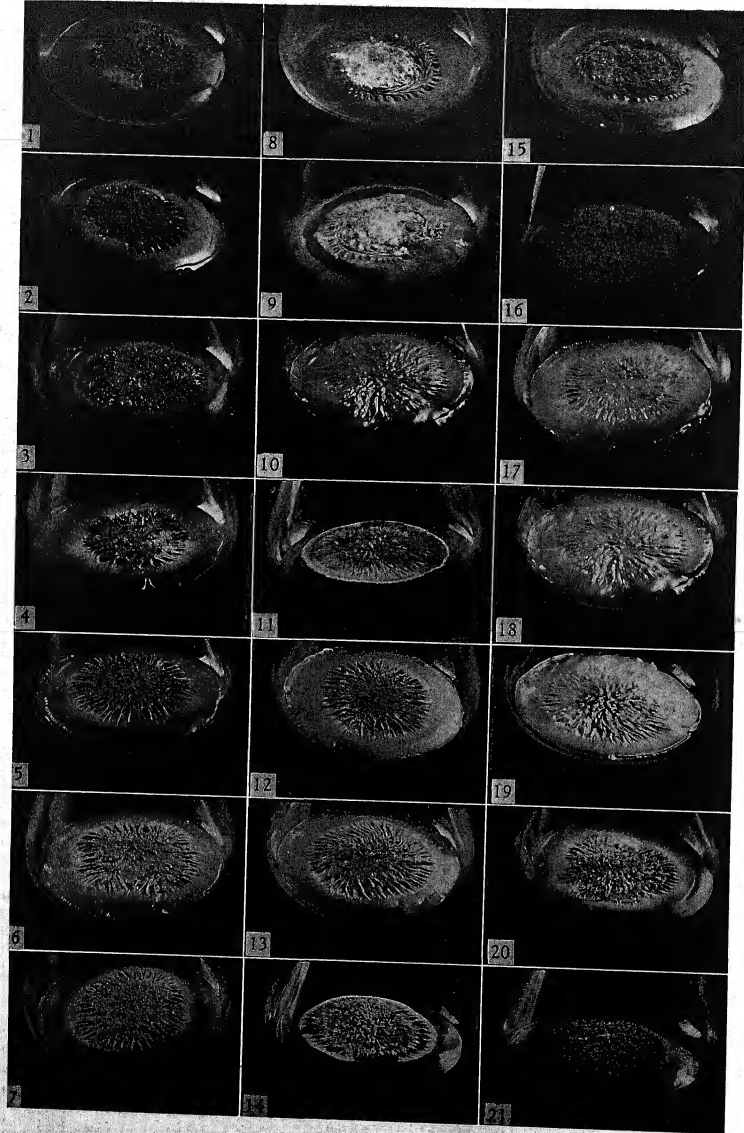
MATERIALS AND METHODS.—The objects of this investigation, covering different races of the loose and covered smuts of oats, were two-fold:

I. *Culture studies* to determine whether: (a) the different races could be distinguished from one another by their cultural characteristics; (b) different methods of isolation would give comparable culture behavior; (c) the different isolations by the same method were similar or dissimilar; (d) the successive transfers in certain isolations would show a constancy in cultural characteristics; (e) the loose and covered smuts were comparable in characteristics and behavior.

II. *Infection experiments* to determine whether: (a) single sporidial isolations were capable of causing infection of the host; (b) the combinations of certain single sporidial cultures, within a given race, would result in infection; (c) interspecific hybridization could be attained by combining certain single sporidial cultures from both the loose and covered smuts; (d) the resulting hybrids would show new morphologic and pathologic characters; (e) new physiologic strains would result from such hybridization.

Studies have been made on eleven physiologic races of the loose smut and seven of the covered smut of oats. Three races of loose smut included two collections each, and one race contained four collections, while two races of covered smut were represented by two and five collections, respectively. The other races of both smuts were single collections. All collections were obtained by Reed from different geographic sources and established as distinct physiologic races by their pathogenic behavior toward selected differential host varieties. Further, Reed (1924a, 1924b, 1927, 1930, 1935), and Reed and Stanton (1932, 1936) demonstrated their constant pathogenicity and, in the instances cited, specific racial groups were determined by the identical behavior of one or more collections.

Three methods were employed for culturing the races on artificial media: (1) dilution, (2) single



chlamydospore, (3) single sporidium. In the first, chlamydospore masses were removed from the center of the smut sorus with a sterile needle and transferred to a 1 per cent copper sulphate solution for 20-24 hours. The usual dilution technique, with potato-dextrose agar, was then followed, and the poured Petri dish plates were incubated at room temperature. Transfers to slants were made from the developing smut colonies within from ten days to two weeks. Equal sized portions of culture were transferred later from the slants to triplicate flask sets of potato-dextrose agar.

Single chlamydospore isolations were made according to Hanna's (1924) method, by which individual, dry chlamydospores were removed from the surface of a sterile microscope slide with a sterile needle and transferred to solid medium on a cover-glass. Incubation took place in Petri dish moist-chambers, and subsequent treatment leading to the triplicate sets was identical with that in the preceding method.

The single sporidial cultures were derived from isolated individual chlamydospores. The progress of germination and development of sporidia on the promycelia of these chlamydospores was carefully observed until the matured sporidia could be isolated by the method and apparatus described by Hanna (1928). Frequently it was found more efficient to draw the single sporidium over the surface of the medium and away from the promycelium to a previously marked point. The isolated sporidia were periodically examined for growth, and when a small colony had developed, it was transferred to a more favorable medium. Triplicate culture sets were finally made for each isolation.

The cultures were grown in Erlenmeyer flasks of 200 cc. capacity which contained 35 cc. of medium. Difco potato-dextrose agar was the standard medium used for all the culture studies; preparation, sterilization, and transferring were done, as nearly as possible, at the same time for each transfer series. All cultures were incubated in a dark room having a rather constant temperature (about 20°C.).

The triplicate flask sets of all isolations were grown for a two months' period. Data were then recorded on the observed cultural characteristics. These characteristics included the following: (1) *Size*. Total growth was determined by measuring the diameter, in centimeters, of each culture and calculating the average size per triplicate set. In figures 1-21 are shown the general form of culture growth for which measurements were obtained. (2) *Color*. Ridgway's Color Standard (1912) served in recording descriptions of this characteristic. The appearance of more than one color, and its location, in a given culture was noted in the description. (3) *Topography*. The

general form or growth type was determined for each culture and expressed in the terminology accepted by other investigators. Figures 10 and 16-19 illustrate the appressed culture type, while the remaining figures are convex. The smooth form is shown in figures 10 and 17-19; rough or granular in figures 3-4 and 7; rugose in figures 5-6 and 17-19, etc.; reticulate in figures 14 and 21; powdery in figures 11 and 14; cottony in figures 8-9; and hairy in figure 16. Marginal borders were usually radially-ridged (fig. 4-6, etc.) but sometimes with striate extensions or entirely striate (fig. 12 and 17-19). (4) *Whirling*. Marginal ridges or striations frequently exhibited tendencies for either clockwise or counter-clockwise directions, and such phenomena were recorded under topography. Typical, well-defined counter-clockwise ridges are shown in figures 8 and 15. (5) *Sectoring*. The appearance in various cultures of spots, large parts of the culture or V-shaped areas which were conspicuously different in color or topography from the remainder of the culture, was noted.

Certain isolations were perpetuated through successive culture generations by transferring small, uniform amounts of culture material from the selected cultures to new triplicate sets. Transfers were also made from numerous sectors or variable cultures in a given triplicate set.

Culture material provided inoculum for the infection experiments according to Dickinson's (1927b) method or modifications of it. Coleoptiles of two-day old seedlings of the oat varieties used were smeared with young culture growth of the desired smut. (Higher infections were not induced by slightly injuring the coleoptiles prior to inoculation.) After inoculation, the seedlings were planted in wax-cups containing sand with a 20 per cent moisture content and incubated in a constant temperature tank (20°C.) until the seedlings emerged. The experiments involving the combination of different single sporidial isolations of a given race and different smut species necessitated mixing the selected cultures and growing them one week prior to seedling inoculation. Reed's (1924b) method was followed for inoculations with dry chlamydospores. All oat seed used had previously been dehulled and treated with formalin (1:320) for ten minutes. After incubation, the inoculated seedlings were transplanted to pots in the greenhouse or field rows along with uninoculated checks. Data were taken at maturity on the number of smutted and normal plants in each inoculation series. The pathologic appearance of the smut was noted and rechecked when microscopic determinations were made of morphologic characters of the smut collected.

EXPERIMENTAL RESULTS AND DISCUSSION.—The results obtained with the different races of both oat

Fig. 1-21. Cultures of races of *Ustilago Avenae* and *U. levis*.—Fig. 1, 8, 15. Variation within a triplicate set of A-I.—Fig. 17-19. Uniformity within a triplicate set of L-I.—Fig. 9, 16; 20, 21. Variations between culture generations of A-I and A-IX.—Fig. 10-11, 13-14. Variations between culture generations of L-I and L-III.—Fig. 5-7, 12-13. Uniformity between individual cultures of different races and collections of *U. levis*.—Fig. 1-6. Similarities between individual cultures from three races of both smuts.—Fig. 6-7, 12-13, 20. Similarities between four races of *U. levis* and one of *U. Avenae*.

TABLE 1. Showing the cultural characteristics of single sporidial isolations in successive culture generations of Race I of *Ustilago Avenae* and *U. levis*, respectively.

(1)	Size in cm.		Topography	
Isolation No.		Color	Surface	Marginal border
A-I:				
1	5.7	Cinnamon-buff, cinnamon	Smooth, rugose-reticulate	Radially-ridged
1-1	4.9	Cinnamon, cinnamon-brown	Rugose-reticulate	Counter-clockwise, radially-ridged
1-2a	5.0	Sayal brown	Stellate, rugose-reticulate	Ditto
1-2b *	5.0	Sayal brown	Compact, rugose-reticulate	"
1-2b-2	5.1	Cinnamon to sayal brown	Granular, rugose-reticulate	Radially-ridged
1-2b-2-3b * ...	5.1	Cinnamon-brown, sayal brown, verona brown	Stellate, rugose-reticulate	Striate
1-2b-2-3a	5.0	Snuff brown, sayal brown, warm sepia	Rugose-reticulate	Radially-ridged
1-2b-2-3a-1 ..	5.3	Cinnamon-buff, sayal brown	Granular, rugose-reticulate	Ditto
I-I:				
5	5.0	Cinnamon-buff, warm sepia	Smooth, rugose-reticulate	Striate, radially-ridged
5-1	5.4	Sayal brown	Rugose-verrucose, smooth-pitted, rugose-reticulate	Counter-clockwise, radially-ridged
5-3b *	5.3	Sayal brown, cinnamon-brown, cinnamon	Rugose-reticulate, rough	Radially-ridged
5-3a	5.2	Cinnamon, sayal brown, snuff brown	Rugose-reticulate, granular, smooth	Irregular radially-ridged
5-3a-3	5.2	Cinnamon, verona brown, sayal brown	Ditto	Radially-ridged
5-3a-3-3	5.5	Warm sepia, cinnamon, wood brown	Rugose-reticulate, radial-rugose	Striate, radially-ridged
5-3a-3-3-2	5.5	Cinnamon-brown, mars brown	Smooth-pitted, rugose-reticulate, rough	Striate, irregular radially-ridged

(1) The first numeral indicates the original isolation; the numerals following show the successive culture generations, being the flask numbers from which the transfers were made; a represents typical cultures, b variant cultures.

* From sector.

smuts may be considered under culture studies and inoculation experiments, the latter based on monosporidial cultures. More detailed studies were made on Race I of the loose and covered smuts, hereafter designated as A-I and L-I.

Culture studies.—The characteristics of the first generation cultures of sixteen single sporidial isolations of A-I were compared, details for one being recorded in table 1. These cultures can be roughly grouped, on the basis of color combinations and certain topographical characteristics, into four classes, as follows: (1) cinnamon-buff, cinnamon, radially-ridged borders; (2) cinnamon-buff, sayal brown, granular, with either radially-ridged or striate borders; (3) cinnamon, pinkish buff, pale smoke gray, with radially-ridged or striate borders; (4) cinnamon, verona brown, warm sepia, radially-ridged borders. There were three additional sporidial isolations which were distinct from one another and the four classes described. The size of cultures varied from 4.8 to 5.9 cm., thirteen being between 5.0–5.2 cm.

First generation cultures from eight single sporidial isolations of L-I were also studied, a description of

one being given in table 1. The classes determined for this race were: (1) cinnamon, stellate-rugose, radially-ridged borders; (2) cinnamon-buff, warm sepia, striate and radially-ridged border; (3) ochraceous-tawny, crater-like, granular, papillate, no obvious border; (4) pinkish buff, pale pinkish buff, wood brown, granular, radially-ridged border. These isolations ranged in size from 4.8 to 6.2 cm.

In both smuts, the isolations comprising the different classes, as well as the exceptions, were not particularly distinctive in their characteristics. The isolation of these sporidia was at random and might well have included a large number with fairly similar basic cultural characteristics, but possessing slight dissimilarities which determined the classes mentioned. Perhaps, if all four sporidia from a given promycelium had been isolated and cultured, more obvious differences would have appeared, unless they were genetically limited to a comparatively few and similar characteristics.

Dissimilarity between the individual cultures of the triplicate sets was generally more common than uniformity in all the sporidial isolation cultures. Isola-

tion No. 1 of A-I and No. 5 of L-I are excellent examples, and such behavior suggests that a continued variation in culture characteristics is taking place. This condition is further noted by the failure of successive culture generations to duplicate the size, color, and topography of the original isolation. Table 1 gives this for one isolation of each smut, respectively. Sectors which appeared were equally unstable and rarely transmitted their traits to subsequent generations. The sectors appear to have resulted from phenotypic variation rather than mutation.

Comparisons were made between the first generation cultural characteristics of seventeen single chlamydospore isolations and eight isolations derived by the dilution method for A-I. In size of culture, the single chlamydospore isolations ranged from 4.6 to 5.6 cm., having no apparent constancy. Size varied from 4.3 to 5.0 cm. in the dilution isolations and exhibited decided variability. The prevailing color was cinnamon-buff, which appeared prominently in four single chlamydospore and three dilution isolations. With the exception of three cinnamon single chlamydospore cultures, all the remaining cultures of both isolation methods generally conformed to pinkish-cinnamon, sayal brown, vinaceous-buff, light ochraceous-buff, and pale pinkish buff. Rugose-reticulate, stellate-rugose, radial-rugose, granular, and smooth were the features of topography most commonly observed and occasionally found repeated in a few cultures. The method of isolation could not be correlated with the appearance of any specific type listed. Similarly, radially-ridged marginal borders were usually present in all the isolations.

The corresponding isolations of L-I were also studied. First-generation cultures from eighteen single chlamydospore isolations and four isolations by the dilution method were compared. The culture size of the single chlamydospore isolations ranged from 4.3 to 5.9 cm. and variability was common, although certain sizes included two to four isolations. In the dilution cultures, size varied between 3.5 and 5.1 cm. with all isolations different. Cinnamon was the characteristic color type for the chlamydospore isolations, but vinaceous-buff and pale ochraceous-buff also appeared frequently. The isolations by this method showed extremely variable characteristics because many other colors and combinations were present. In the dilution isolations, sayal brown was characteristic of two, while the other two were totally distinct. In topography, all the isolations were rugose-reticulate, stellate-rugose, granular, smooth, or combinations of these types. Radially-ridged marginal borders were present in the majority of isolations.

In each race of both oat smuts, the single chlamydospore and dilution cultures exhibited behavior similar to those noted for the monosporial cultures. An identical analysis of the isolations derived in the former methods was accentuated by a consideration of the combined cultural characteristics and indicated that dissimilarity was more prevalent than similarity among the numerous isolations.

A brief description of a single sporidial isolation, carried by transfer through several culture generations, is given for both A-I and L-I in table 1. An analysis of all the cultures of A-I, including those not recorded in the table, indicates that dissimilarity among the triplicate cultures of a given set is more frequent than uniformity. Figures 1, 8, and 15 show this for dilution isolation No. 3. In eighty-five different triplicate sets, fifty-three could be classed as dissimilar while thirty-two were rather uniform. Thirty original isolations of L-I were made, from which forty-two triplicate sets show dissimilarity within the individual sets and twenty-eight uniformity. Figures 17-19 illustrate uniformity between the cultures of a triplicate set of dilution method No. 2.

Comparisons were made between the various culture generations of A-I, ranging from three to nine, for two isolations from each of the three methods. Also, five to nine generations of L-I isolations were studied. The results generally show that the triplicate sets were dissimilar from one generation to another regarding size, color, and topography. Certain exceptions are found, but rarely were they followed by complete repetition in all subsequent generations. Dilution isolations No. 2 and 3 (fig. 16) of A-I illustrate permanency in transmission of the hairy type topography. An analysis of all the component cultural characteristics in any set establishes a clearer view of the existing dissimilarities than does a comparison of the individual characteristics. A typical example of the striking differences between the second and third culture generation is shown in figures 9 and 16 for dilution isolation No. 3 of A-I, and figures 10-11 for dilution isolation No. 2 of L-I.

The two races of both smuts, when compared, provided interesting points. The number of sets showing uniformity or dissimilarity within them, individually, appeared in the same proportion for both smuts and averaged approximately 60 per cent for variability. Cinnamon, cinnamon-buff, cinnamon-brown and sayal brown were the common basic colors. The topographic characteristics showed both as predominantly rugose-reticulate, stellate-rugose, radial-rugose, granular, or smooth. Finally, neither the individual culture characteristics nor the combined characteristics were generally capable of demonstrating similarities between the successive generations within each smut; the method of isolation appeared to have no effect on this behavior. Certain comparative cultural similarities could be established between A-I and L-I by the random selection of individual cultures from their isolation sets.

Ten other races of the loose smut were studied for their cultural behavior by the dilution method. Dissimilarities were apparent within the triplicate cultures of a given set as well as between different isolations in the same race, while uniformity in all instances was of minor significance. The dissimilarities earlier noted between successive culture generations were again conspicuous in these races, and were emphasized by comparing the complex of cultural

characteristics. The second and third generations of Race IX clearly illustrate this behavior in figures 20-21. First generation comparisons were made between ten single chlamydospore cultures of Red Rust-proof Race (X^a), a race distinct in its pathogenicity. Size ranged from 4.8 to 5.3 cm. and topographic characteristics were equally variable. Each isolation was quite distinct, but the degree of dissimilarity ranged from slight to decided on the basis of individual characteristics. Selections of one or more individual cultures from many of these races demonstrated definite differences between them (fig. 2, 8, 20 with Races III, I, IX). Further, selections of certain cultures clearly indicated similarities between the races, as is shown in figures 1-3 for Races I, III, and IV, respectively.

The cultural behavior of six additional races of the covered smut strikingly paralleled the results just stated. Two races having well defined pathologic behavior provided numerous single chlamydospore isolations. In Race II, nineteen isolations were compared for first generation characteristics, while, in Race VI there were twenty such isolations. The comparative results confirm those obtained with other isolations by this method. However, five isolations of Race II were distinguished by chamois and honey yellow colors, but the remaining characteristics were noticeably variable. Dissimilarities between successive culture generations are demonstrated by the second and third generations of Race III in figures 13 and 14. Selection of particular cultures from among the different races provided a series exhibiting uniformity between several of them, and figures 5-7 and 12-13 show this relationship between Races I^a, I, VI, IV, and III, respectively. Dissimilarities, likewise, may be shown between Races I, V, and IV in figures 10, 4, and 12. In none of the races of either smut was the transmission of characteristics, observed in sectors or extremely variable individual cultures, found to take place.

The existence of cultural similarities between the races of either the loose or covered smut may also be used to include both smuts when individual cultures are selected for comparison. Such a series, comprising Races I, III, and IV of the loose smut and V, I^a, and I of the covered smut, is illustrated in that sequence in figures 1-6. Another series demonstrating similarities is shown in figures 6-7, 12-13, and 20 in which cultures of Races I, VI, IV, III of *Levis* and IX of *Avenae* are compared.

Dissimilarities between the different isolations, the cultures comprising triplicate sets, and the successive culture generations have been shown to be characteristic of all the single chlamydospore and dilution isolations. The original isolations from both these methods comprised several haploid sporidia which, no doubt, contained factors for certain cultural characteristics. Consequently, the mixture of these sporidia, each of which might produce different cultural traits, provides for an indefinite number of culture types. This may be apparent within a triplicate set or may

account for the continuous appearance of new characteristics in successive culture generations. The appearance of sector-like areas in certain cultures is very likely due to the expression of cultural characteristics by one of the component sporidia in a localized area of the culture. However, this explanation is limited since sectoring has been observed in monosporidial cultures. Kinsley (1933) states that single chlamydospores were unreliable for the determination of cultural characteristics of a given race since constancy proved to be an exception and dissimilarities within sets were often as great as between the different forms used.

Dickinson (1928, 1931) did not study the behavior of chlamydospore cultures of the covered smut, but with isolated single sporidia found that the culture characteristics segregated according to one of three ratios. This segregation occurred in either the first or second reduction divisions and, according to him, was complete, since successively isolated sporidia from the same promycelial segment never produced new cultural types. The characteristics remained permanent according to his methods, which did not include transfers through successive culture generations. Hüttig (1931) also found segregation of cultural characteristics to occur in the loose smut. In 1931 and 1932, Holton reported that segregation of cultural characteristics might occur in the first, second, or even later divisions of the fusion nucleus, while segregation for sexual characteristics was limited to the first or second divisions. Culture variants which he observed were considered the result of delayed or extended reductions. My findings are not in complete agreement with those of Dickinson. If the four classes of culture types mentioned for A-I and L-I had been strictly different, then segregation would appear to have resulted for certain characteristics. This was not established, because such characteristics lacked permanency in the triplicate sets and in their successive culture generations, for certain isolations. Further, Holton's interpretation of delayed or extended segregation does not explain these behaviors. The haploid sporidia should contain the reduced number of cultural factors, and these factors should theoretically remain unaltered, regardless of the number of vegetative or cultural generations. Therefore, continued segregation beyond the third division of the fusion nucleus or any other present facts do not account satisfactorily for the unstable condition of the cultural characteristics in these monosporidial cultures.

Rodenhiser (1926, 1928) distinguished several races of the loose and covered smuts by means of multi-chlamydospore cultures in which the differentiating characteristics were obtained only on the proper medium. Certain races exhibited differences which were frequently greater than those apparent between the smut species. He also observed similar behaviors with the loose smut of barley and wheat. The results of the present investigation do not substantiate his work. The data indicate considerable variability

which is particularly noticeable in the single chlamydospore and dilution isolations, and such variability eliminates the determination of a definite and permanent culture type for any given race studied. Further, cultural dissimilarities between different races and species can be obtained, but only by a careful selection of individual cultures. For example, selections of single cultures from A-I, A-I^a, A-II, A-VI, A-VII, A-IX, and from L-I, L-IV, L-II, L-III, L-V, and L-VI gave two series demonstrating such dissimilarities. The cultures selected were not necessarily the most common or typical for their respective races, and entire triplicate sets were seldom included in these comparisons. The constant reduplication of culture characteristics has never been found, and consequently, the dissimilarities mentioned above are neither permanent nor dependable in distinguishing one race from another. Finally, many races appear to be similar to one another in their cultural traits; figures 5-7 and 12-13 show this for L-I^a, L-I, L-VI, L-IV, and L-III, respectively. Corresponding comparisons were noted in the loose smut, and again, such results were obtained solely by selecting single cultures. Therefore, the failure of all cultures from a given race to show uniformity makes it impossible to differentiate between the smut races used.

Culture characteristics have also been found unreliable for distinguishing between different races, forms, or strains by Eddins (1929) in *Ustilago Zeae*; Rodenhiser (1932) in *Sphaelotheca Sorghi*; Kienholz and Heald (1930), Flor (1933), Melchers (1934), and Becker (1936) in *Tilletia laevis* or *T. Tritici*. Variabilities and mutations in cultures have been noted by Christensen and Stakman (1926), Hanna (1929), Stakman, Christensen, Eide and Peterson (1929), Stakman, Tyler, Hafstad, and Sharvelle (1935) for *U. Zeae*; Rodenhiser (1928) for *U. nuda*; Ficke and Johnston (1930) for *S. Sorghi*.

Rodenhisser reported similarities between certain races in two closely related smuts. Such similarities were observed in this work but only by the selection methods cited above. These similarities were often greater than those observed between the different races of the loose or covered smut.

Inoculation studies.—A-I and L-I are distinguished by three important characteristics. They differ in the symptoms or structural changes on the infected host plant at maturity. In A-I the oat spikelets usually are entirely destroyed and replaced by black, dusty masses of chlamydospores, while L-I destroys the kernel and only partially the glumes. They are unlike in morphologic characteristics, the A-I chlamydospores being from 5-11 μ in diameter with echinulate walls and the L-I spores 5-9 μ with smooth walls. They differ in host infection, which is distinctly a pathogenic characteristic and is not accompanied by distinguishing symptoms or morphologic characteristics. A-I causes complete infection of Gothland variety of oats, but Monarch is fully resistant. However, Monarch is completely susceptible to L-I, while Gothland is resistant.

Certain difficulties were encountered in using sporidial culture material for inoculum. Uniform smearing of the oat coleoptiles could be obtained only when the cultures had a soft-viscid consistency. Differences in consistency made it rather difficult to obtain uniform mixtures when sporidial cultures were combined, and great care was necessary to prevent the inoculum from drying out following inoculation. The variable germination of the oats required careful selection for coleoptiles with uniform length. The infection ability of all culture materials was unknown, and the time at which inoculation was made might not have coincided with the time at which the coleoptile was susceptible to penetration. Compatibility, or the positive reaction between paired monosporidial cultures of opposite "sex," was not determined prior to inoculation. Some investigators have observed that fusion, or positive reaction, in vitro occurs only between paired compatible cultures and that host infection results only from such pairings. Consequently, infection data were relied upon for evidence of such compatibility in this work.

In 1933, inoculation experiments with sixteen different single sporidial cultures of A-I gave no infection on Gothland, its susceptible host variety, and six corresponding cultures of L-I failed to infect its susceptible host, Monarch. Therefore, the inability of single sporidial cultures to produce infection on host varieties of known susceptibility was shown.

However, when thirteen different monosporidial cultures of A-I were paired in thirty-five combinations, typical loose smut infections were obtained on Gothland in twenty-three cases. Thus, two fusion or "sex" groups were determined. These results corroborate the evidence of other workers concerning the heterothallic nature of the sporidia and the necessity for paired opposite "sexes" (sporidia) to produce infection. Inoculation of the host with paired monosporidial cultures of the same "sex" explains the absence of infection on Gothland with twelve combinations. The determination of infective behavior of the few monosporidial cultures of L-I on Monarch was limited to three combinations whose negative results indicated the "sexual" similarity of the cultures involved.

Three monosporidial cultures of L-I were paired in numerous ways with thirteen of A-I, and the thirty-nine resulting combinations were used to inoculate both Monarch and Gothland. From these, there were only two different combinations which gave infection, and then solely on Monarch. Furthermore, just one plant out of five showed infection, and the smut had the symptoms and morphology of the loose type in each case. This type of smut is significant because Monarch normally is highly susceptible to L-I and rarely, if ever, to A-I. It was apparent that the two combinations inducing infection were each composed of two monosporidial cultures having compatible "sex" reactions. Since these pairings brought together two smut races from different species, positive reaction may be assumed to

indicate hybridization between them. This point is illustrated further by the production on Monarch of new types of smut having the symptoms and morphologic characteristics of the loose smut but the pathogenic qualities of the covered smut. Such results indicate that the loose type symptoms and morphology are dominant over the covered type but that the covered type pathogenicity is dominant over the loose. Data obtained from subsequent infection tests will be used for further consideration of the hybrid nature of these original combinations.

The failure of infection by more combinations may be due to the difficulties encountered with sporidial inoculum, discussed earlier. In addition, the comparatively small number of seed used for each variety was not sufficient to compensate for any limiting factor in the inoculation procedure.

The two combinations producing infection comprised pairings between four entirely different monosporidial cultures. Combination A contained monosporidial culture No. 6 of A-I and No. 3 of L-I, while combination B had No. 12 of the former and No. 5 of the latter smut species. The smut which they produced on Monarch consisted of numerous F_1 chlamydospores, which were used as dry spore inoculum on a series of host varieties in 1934. The infected plants produced F_2 chlamydospores. In fact, each smutted host yielded a population of different F_2 's, since the inoculum used was not from a single F_1 chlamydospore. Theoretically, the F_2 chlamydospores contained all the possible combinations between the symptoms, morphologic, and pathogenic characteristics derived from the parent smuts in hybridizing. In 1935 and 1936 the same procedure was continued, using inoculum of different F_2 and F_3 chlamydospore populations.

The host varieties used may be distributed into three classes: (1) varieties commonly infected by both smuts: Canadian, Danish Island, Early Champion; (2) varieties susceptible to A-I but not to L-I: Gothland, Monarch Selection, Seizure, Danish, Rossman; (3) Monarch, susceptible to L-I but not to A-I.

It may be seen that a complex relationship was introduced, involving the reactions of the chlamydospores against hosts which differed in resistance and susceptibility. The use of chlamydospore populations offered numerous possibilities for the production of different smut types, quite distinct from that found in any single chlamydospore of a given population. This could result from the fusion of sporidia from different chlamydospores or two smut types might have existed within a chlamydospore population but one was eliminated because the particular host was resistant to it. Such examples demonstrate the impracticability of predicting the appearance of a definite smut type on a given host, at least in the early generations. It is obvious that such a situation prevented observations on the segregation of symptoms and morphologic characteristics in these hybrids.

The outstanding results from infection studies originating from combinations A and B are presented

below. Monarch was infected with both combinations in 1933, the chlamydospores obtained being used in 1934 to inoculate the varieties indicated. These varieties are primarily the ones showing the greatest differential effect with the two smut types. In the fractions, the denominator gives the total number of plants inoculated while the numerator shows the number infected. The first capital letter designates symptoms; the second, morphologic characteristics, with A representing the loose type and L the covered type smuts. A superscript 1 appears for material difficult to determine, while 2 is used for variable material which more definitely resembles the type given. Various recombinations of the characters for symptoms, morphology, and pathogenicity resulted in the production of certain new smut types in the F_4 .

COMBINATION A:		1934— F_2	1935— F_3	1936— F_4
Gothland	1/15	L L		
			Gothland 14/16	L L
				Gothland 16/20 L L
				Monarch 12/18 L L
	Monarch	4/17	L L	
			Gothland 16/18	L L
			Monarch 15/18	L L
	Rossman	14/17	L L	
			Gothland 16/16	L L
			Monarch 4/10	L L
Monarch	4/15	L L ¹		
			Gothland 0/10	- -
			Monarch 10/15	L L ²
			Gothland 0/18	- -
			Monarch 11/15	L L ¹
	Seizure	1/11	L L ²	
			Gothland 13/16	L L
			Monarch 5/11	L L
			Seizure 10/11	L L
	Rossman	1/13	L A ²	
Canadian	3/4	A A ¹		
			Gothland 2/11	A ¹ A
			Gothland 15/19	A ¹ A
			Monarch 0/15	- -
	Monarch	2/10	A A	
			Gothland 0/19	- -
			Monarch 17/20	A A
	Danish Island	2/15	A A	
			Gothland 4/13	L ² A ²
			Gothland 13/14	L ¹ A
Monarch			Monarch 0/13	- -
			Monarch 3/10	A A
			Gothland 9/19	A ¹ A
			Monarch 12/19	A A

1934—F ₂			1935—F ₂			1936—F ₄			1934—F ₂			1935—F ₂			1936—F ₄		
Early Champion	13/14	A ¹ A	Gothland	4/10	A A	Gothland	13/13	A A	Gothland	2/7	A ¹ A	Early Champion	11/14	A A	Gothland	11/11	A ¹ A ¹
			Monarch	1/14	A A	Gothland	0/11	—	Monarch	3/7	A ¹ A	Monarch	1/10	A ¹ A ¹			
						Gothland	0/12	—									
						Monarch	15/15	A A ¹									
Monarch Select.	1/15	L L ¹	Early Champion	3/6	L L	Gothland	2/17	L L									
						Monarch	0/7	—									
			Gothland	1/12	L ¹ L ¹	Gothland	15/15	L ² L									
						Monarch	0/12	—									
			Monarch Select.	3/12	L ¹ A ²	Gothland	11/20	L L									
						Monarch	1/17	L ¹ L									
						Monarch Select.	8/18	L L									
COMBINATION B:																	
Gothland	1/14	L L	Gothland	6/14	L L ²	Gothland	7/18	L L									
						Monarch	0/20	—									
			Monarch Monarch Select.	0/13 4/12	— L L ¹	Gothland	3/20	L L									
						Monarch	0/18	—									
						Monarch Select.	1/19	L L ¹									
			Seizure	5/11	L ¹ L	Gothland	0/19	—									
						Monarch	0/16	—									
						Seizure	14/14	L ¹ L									
			Rossman	5/13	L L	Gothland	3/18	L L									
						Monarch	0/18	—									
						Rossman	9/18	L L									
Monarch Canadian	0/14 13/15	— A A	Gothland	2/5	A A	Gothland	17/19	A A ¹									
						Monarch	6/15	A A ²									
			Monarch	8/10	A A	Gothland	0/18	—									
						Monarch	11/12	A A ²									
Danish Island	4/15	A A	Gothland	9/9	A A	Gothland	19/19	A A									
						Monarch	5/16	A A									
			Monarch	3/13	A ¹ A	Gothland	5/5	A ¹ A									
						Monarch	8/8	A ¹ A									
Early Champion	10/15	A A	Danish Island	4/8	A A	Gothland	0/16	—									
						Monarch	3/7	A ¹ A ²									

Both combinations A and B have shown certain similarities in the infection tests reported. Some new smuts having the symptoms and morphology of the covered type, with their percentages of infection in the F₄, follow:

1. Gothland, 100%; Monarch, 0%
2. " 39%; " 0%
3. " 12%; " 0%
4. " 89%; " 83%
5. " 100%; Rossman, 80%; Monarch, 40%
6. " 94%; " 100%; " 0%
7. " 17%; " 50%; " 0%
8. " 55%; Monarch Sel., 44%; " 6%
9. " 15%; " " 5%; " 0%
10. " 0%; Seizure 100%; " 0%

The types having the symptoms and morphology of the loose smut were:

1. Gothland — 0%. Monarch — 100%
2. " — 0%, " — 92%
3. " — 0%, " — 85%
4. " — 0%, " — 43%
5. " — 45%, " — 63%
6. " — 100%, " — 100%

Numerous other types were noted in which both Gothland and Monarch were incompletely infected in varying degrees by smuts having the covered or loose characteristics.

These results indicate the production of new smuts showing various symptomatic, morphologic, and pathogenic characteristics not observed previously on the hosts used. Further, the new smut types are apparently the result of the recombinations possible between the typical characteristics from both A-I and L-I. In several cases Gothland was found to be highly infected with different covered smut types while Monarch remained resistant. Since Gothland is normally resistant and Monarch completely susceptible to the L-I smut, these data point to a recombination of characters, the A-I type of pathogenicity having combined with the symptoms and morphology of the L-I smut. Other new smut types appeared in which both varieties were infected in varying degrees, and, generally, Gothland showed the highest percentage of smut, although a few types infected both Gothland and Monarch about equally. Pathogenicity was incomplete and probably the result of entirely new combinations for this characteristic. A most unusual covered type smut was the one caus-

Both combinations A and B have shown certain similarities in the infection tests reported. Some new smuts having the symptoms and morphology of the covered type, with their percentages of infection in the F₄ follow:

1. Gothland, 100%; Monarch, 0%
2. " 39%; " 0%
3. " 12%; " 0%
4. " 89%; " 83%
5. " 100%; Rossman, 80%; Monarch, 40%
6. " 94%; " 100%; " 0%
7. " 17%; " 50%; " 0%
8. " 55%; Monarch Sel., 44%; " 6%
9. " 15%; " 5%; " 0%
10. " 0%; Seizure 100%; " 0%

The types having the symptoms and morphology of the loose smut were:

1. Gothland — 0%. Monarch — 100%
2. " — 0%, " — 92%
3. " — 0%, " — 85%
4. " — 0%, " — 43%
5. " — 45%, " — 63%
6. " — 100%, " — 100%

Numerous other types were noted in which both Gothland and Monarch were incompletely infected in varying degrees by smuts having the covered or loose characteristics.

These results indicate the production of new smuts showing various symptomatic, morphologic, and pathogenic characteristics not observed previously on the hosts used. Further, the new smut types are apparently the result of the recombinations possible between the typical characteristics from both A-I and L-I. In several cases Gothland was found to be highly infected with different covered smut types while Monarch remained resistant. Since Gothland is normally resistant and Monarch completely susceptible to the L-I smut, these data point to a recombination of characters, the A-I type of pathogenicity having combined with the symptoms and morphology of the L-I smut. Other new smut types appeared in which both varieties were infected in varying degrees, and, generally, Gothland showed the highest percentage of smut, although a few types infected both Gothland and Monarch about equally. Pathogenicity was incomplete and probably the result of entirely new combinations for this characteristic. A most unusual covered type smut was the one caus-

ing complete infection of Seizure but no infection on Gothland and Monarch. Gothland and Seizure normally have the same pathogenic reactions to A-I and L-I; but the complete susceptibility of Seizure, and not Gothland, indicates an entirely new recombination of characteristics having a high degree of specialization towards one host variety. Certain covered smut types attacked Monarch but not Gothland. Such smut types were characteristic of the L-I parent and no doubt resulted from a segregation of the pure parental types. New loose smut types were also recognized and several infected Monarch while Gothland proved resistant. The degree of infection obtained on Monarch ranged from moderate to complete. Varying degrees of pathogenicity were, therefore, produced by the new combinations incorporating the symptoms and morphology of A-I with the pathogenic behavior of L-I. Several loose types caused infection on both Gothland and Monarch which was generally incomplete. The recombinations of characteristics found in these types showed variability in pathogenicity only. One new loose smut type was particularly noteworthy in that Gothland and Monarch were both completely susceptible to it. The new combination of characteristics responsible here has provided a distinctive pathogenic feature and has not occurred in any other new types. Gothland gave 100 per cent infection with certain loose smut types, whereas Monarch remained resistant. This behavior is typical of A-I on these varieties and may be considered the result of the segregating out of the parental type.

Strains having certain similarities in behavior appear to have been established among the infection tests of both combinations A and B. In A, a new smut produced the covered type on Gothland for three generations, with F_3 and F_4 infections of 88 and 80 per cent, respectively. Gothland was infected by three generations of a covered smut type of B in which 43 and 39 per cent smut was obtained in the F_3 and F_4 , while Monarch was resistant. Such behaviors prove this a significantly new smut type. Rossman was infected with a covered type, of combination A, and constant pathogenicity was demonstrated by the F_3 and F_4 generations with 82 and 80 per cent smut. A similar type of smut, for three generations, severely infected Monarch and in the F_3 and F_4 showed 67 and 73 per cent susceptibility, while Gothland remained resistant. This strain seems to be typical for the parental L-I characteristics. Finally, in B, a new smut infected Monarch 80 per cent in the F_3 and 92 per cent in the F_4 . This smut was typically loose, but Gothland was not susceptible to it in the F_4 . The constancy of pathogenicity and resistance of Gothland separates this type as a distinctly new one.

Other new smuts may possibly be distinct strains but are not considered, because the data are limited to two generations and the percentages of infection found on the host varieties were not constant during those periods. The presence of doubtful pathologic

and morphologic characteristics suggests that a continued segregation is taking place. This is emphasized by lack of constancy in the pathogenic behavior during successive generations. However, it is probable that many of the new smut types appearing in the F_4 would become constant if tested in later generations. The ability to attack one important differential host variety and not the other, combined with the symptoms and morphologic characteristics of the smut to which each variety is normally resistant, strongly indicates the establishment of distinctly new physiologic races. This point may be further stressed by considering the behavior of known physiologic races which show uniform symptomatology and morphology but differ by specific pathogenic behavior toward differential host varieties. The new physiologic races produced here conform to the standards for identification of such specialized forms in the oat smuts.

Hanna and Popp (1930, 1931) accomplished hybridization between these oat smut species, obtaining loose smut symptoms and echinulate spores. No mention was made of differential host reaction to their paired sporidial cultures. Holton (1932) also reported hybridization between the two oat smuts in which varying infections from different sporidial combinations were obtained on Anthony oats, a variety highly susceptible to both smuts. Echinulate spores and loose type symptoms were noted in the smut produced, the latter varying considerably. My results confirm the preceding studies to some extent. The symptoms and morphology of the hybrid smut agree, but the use of Gothland and Monarch as hosts indicates a differential pathogenicity by the hybrid sporidial cultures not reported before. The infection of Monarch but not Gothland has already been discussed.

Holton (1936a) made interspecific crosses between the identical smut races covered in this paper. Pathogenicity tests were made on Gothland, Monarch, and Anthony oat varieties with F_1 chlamydospores, followed by F_2 selections for the covered type on Gothland and the loose type on Monarch. The F_2 covered type segregates with smooth spores were considered to be morphologically pure lines, while the loose type segregates having echinulate spores were either homozygous or heterozygous. Pathogenicity was not necessarily associated with the morphologic characteristics. The F_2 covered type gave 22 per cent infection on Gothland and 21 per cent on Monarch; the F_2 gave 92 and 45 per cent, respectively. Holton concluded that a new pathogenic strain had been produced. In the present investigations the morphologic smut types appearing in the F_3 did not completely substantiate Holton's observations. Combination A contained two covered type smuts which in the F_3 and F_4 produced covered and loose type infections, both in symptoms and morphology. The loose types usually were found on varieties completely susceptible to both A-I and L-I, while the covered types appeared on hosts either resistant or susceptible

to L-I. The F_2 loose smut types produced both symptomatic and morphologic loose and covered smuts in the F_3 and F_4 . In one case all three generations showed only the loose smut characteristics. The homozygous or heterozygous types produced from the F_2 loose smuts are in agreement with Holton's studies, but an explanation is needed for the results noted in the covered smut generations. The production of the loose and covered types from the F_2 covered smuts is no doubt an interaction between the inoculum, consisting of populations of chlamydospores in a given generation, and the differential reactions of the host varieties. The expression of certain symptoms and morphologic characteristics would depend upon the straining effect by the infected hosts. For example, in an F_2 chlamydospore population factors for both loose and covered types might be present. The varieties susceptible to either the loose or covered type, but not both, would tend to eliminate the smut type to which it was resistant. Again, through the process of recombination in hybridizing, these same varieties might be infected with smut bearing factors for both the loose and covered types, but would show only the symptoms and morphologic characteristics of the type normally producing high infection. The several differential hosts used would tend to strain out smut types which did not have the normal pathogenic behavior toward specific varieties. Exceptions to this would be in the cases of new combinations having the symptoms and morphologic characteristics of the loose type with the full pathogenicity of L-I, and the covered type with A-I infection behavior. Thus, the varieties commonly susceptible to both smuts, theoretically, would permit all the characteristics to be carried over. The dominance of the loose type could mask the presence of the covered type, but on further inoculation tests both the loose and covered types would appear.

With combination B, a single F_2 covered type demonstrated its purity for three generations while the F_2 loose types were either pure or segregating lines.

Numerous new races have been developed in these tests, their symptoms and morphology being either the loose or covered type, or types difficult to determine. These races have differed primarily in their pathogenicity, which has shown striking effects on the differential host varieties used. Some new races were constant for their respective characteristics during two or three generations.

SUMMARY

Single sporidial, single chlamydospore, and dilution cultures were studied for eleven physiologic races of

the loose smut (*Ustilago Avenae*) and seven of the covered smut (*U. levis*) of oats. The size, color, and topography of the colonies were determined for 274 culture sets of the former and 307 of the latter smut.

The individual cultures comprising the different triplicate sets were generally dissimilar. Approximately 60 per cent of all the triplicate sets of both smuts showed dissimilarities.

Successive culture generations failed to remain constant in characteristics, regardless of the isolation method. The dissimilarities observed between generations sometimes fell within a range of characteristics for a given line or race.

The different single chlamydospore and dilution cultures of respective races were usually distinct from one another, while the single sporidial cultures could be roughly grouped into four classes.

Proper selections of individual cultures from among the different races of either smut could be made to show similarities. In a like manner dissimilarities also could be shown. Such dissimilarities sometimes were not as pronounced as those found between differential isolations in the same race.

Similarities or dissimilarities between the races of both the loose and covered smuts were obtained by proper selection of cultures.

Cultural characteristics did not offer an established means for a definite or permanent identification of the races of loose or covered smuts studied.

Hybridization between A-I and L-I was accomplished with two combinations of paired monosporidial cultures. Monarch was infected with a symptomatic and morphologic loose smut type. Gothland remained resistant.

At least sixteen new smut types were produced which exhibited recombinations of factors for symptoms, morphology, and pathogenicity. Determinations of these types were made during successive inoculations of differential host varieties with populations of hybrid chlamydospores from each generation.

Constant strains appear to have been established by some of the new smut types.

Hybridization between A-I and L-I has produced many new and distinct types of smut. These new types may be considered as new physiologic races by their behavior on differential oat varieties. Such results suggest the possible origin of new specialized races.

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A NEW CHYTRID GENUS: NEPHROCHYTRIUM¹

J. S. Karling

IN CONNECTION with my observations of *Diplophlyctis* in 1928 another chytrid was encountered in dead internodes of *Nitella* and *Chara* which was then believed to relate to the same genus. An analysis of the data and drawings made at that time, however, shows that it is quite distinct in method of development and thus merits consideration as a new fungus. Publication of these data has been delayed in hopes of finding the organism again and completing my observations on certain phases of its structure and development. Unfortunately, it has not been found since its initial appearance, but I am none the less bringing my early data to the attention of mycologists at the present time with the hopes that they will stimulate a more extensive search for this chytrid.

The sporangia and zoospores are strikingly similar in appearance to those of *D. intestina*, but the development of the former structure appears at present to be fundamentally different. Whereas in *D. intestina* the sporangia and resting spores are formed as enlargements of the germ tube (Karling, 1930), in the present species they develop as protuberances or outgrowths from a transverse, fusiform, spindle-shaped, medianly constricted apophysis. Furthermore, occasional intercalary, spindle-shaped swellings occur in the rhizoidal system, while the zoospore case becomes thickened, amber in color, and persists on the surface of the host cell as an appendage to the sporangia and resting spores. These characteristics distinguish this fungus from any of the known chytrid genera, and I am tentatively proposing a new genus, *Nephrochytrium*, to include it. This generic name is chosen with the view of emphasizing the characteristic shape of the sporangia and resting spores, and since the zoospore case is persistent as an appendage, I am naming the type species *N. appendiculatum*.

***Nephrochytrium* gen. nov.** Thallus intramatrix, monocentricus, eucarpicus. Zoosporangia forma diversa, apophyside atque papillis vel tubulis longitudine variis praedita; apophyside excreta et pariete maturitate disjuncta. Systema rhizoidorum valde ramosorum crassa, fusiformibus, intercalariis, incrementis aliquando longius facta lata; zoosporae uniguttulatae atque cilio posteriore praeditae; sporae perdurantes forma diversae, apophyside atque pariete crasso praeditae; apophyside excretae; germinatione incompleta.

***N. appendiculatum* sp. nov.** Fungus saprophyticus; zoosporangium numerosum, hyalinis, levibus subsphaericis, complanatis, depressis, plerumque paullo reniformibus $8 \times 14 \mu$ usque ad $18 \times 20 \mu$, papillis vel tubulis osteolaribus 1-3 longitudine variis ornatis; zoosporis hyalinis, sphaericis $3.5-4.5 \mu$, globulo magno pellucido refracto ornatis; cilio circa 40μ longo; vagina zoospori muris crassesscentibus sucineo-colora-

tis et in superficie cellulae hospitis post germinationem persistenti; apophyside elongato transversum plerumque fusiforme et in medio constricto; rhizoideis modo ramosorum extremorum apophysidis nascentibus et aliquando 600μ diametro extensibus et saepe singulis usque ad $5-6 \mu$ in diametro; sporis pedurabibus levibus pallidis. ad atro-sucineo-coloratis plerumque plusminus reniformibus complanatis depressis aliquando obpyriformibus $10 \times 18 \mu$ usque ad $17 \times 26 \mu$; crasse munitis, uno vel pluribus globulis refractis ornatis; germinatione ignota.

***Nephrochytrium* gen. nov.** Thallus intramatrix, monocentricus, eucarpicus. Zoosporangia variously shaped, apophysate, with one or more exit papillae or tubes of varying length; formed as an outgrowth from the apophysis and delimited from the latter by a cross wall at maturity. Rhizoidal system coarse, richly branched, and extensive, with occasional elongate, spindle-shaped, intercalary enlargements. Zoospores uniguttulate, posteriorly uniciliate. Resting spores variously shaped, apophysate, thick-walled; formed as an outgrowth from the apophysis; germination unknown.

***N. appendiculatum* sp. nov.** Zoosporangia numerosa, hyalina, smooth, sub-spherical, flattened depressed, usually somewhat kidney-shaped, $8 \times 14 \mu-18 \times 30 \mu$, with 1-3 exit papillae or tubes of varying length. Zoospores hyaline, spherical, $3.5-4.5 \mu$, with a large clear refractive globule; cilium approximately 40μ long; zoospore case becoming thick-walled, amber colored, and persisting on the surface of the host cell after germination. Apophysis elongate, transverse, usually spindle-shaped and medianly constricted; rhizoids arising as branches of its ends, extending occasionally over a radius of 600μ and often attaining a diameter of $5-6 \mu$; intercalary swellings $4-8 \mu$ in diameter. Resting spores smooth, light to dark amber, usually somewhat kidney-shaped, flattened, depressed, occasionally obpyriform, $10 \times 18 \mu-17 \times 26 \mu$, thick-walled with one or more refractive globules.

Saprophytic in cells of *Chara coronata*, *C. delicatula*, *Nitella flexilis*, and *N. gracilis* in New York City.

DEVELOPMENT OF THE THALLUS.—The zoospores come to rest on the surface of the algal cell and develop a germ tube which penetrates the wall of the host. They may sometimes germinate in the water outside, as is shown in figures 2B and 2C, and in such cases the germ tube appears to be fairly thick and conspicuous. Germination in situ is rather common in the cases of zoospores which are trapped in the sporangium and fail to escape, as is shown in figure 2D. As soon as it enters the lumen of the cell the germ tube branches more or less dichotomously (fig. 2C) and thus forms the rudiments of the apophysis and rhizoidal system. These branches

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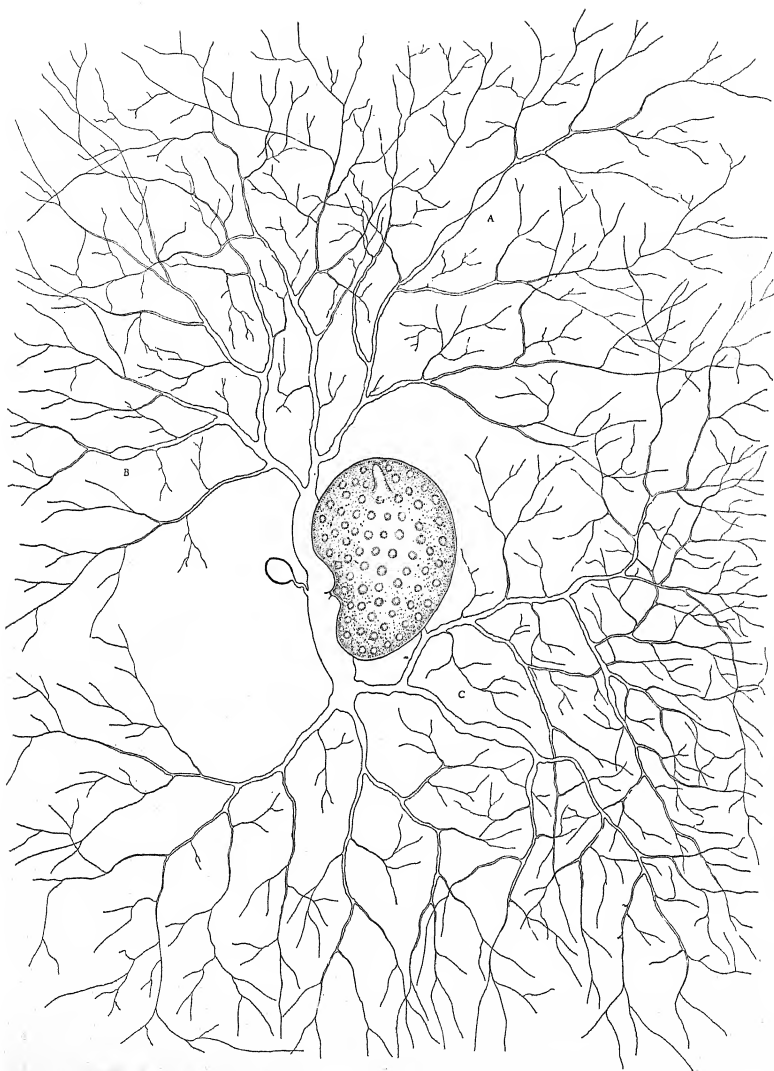


Fig. 1 showing the structure of the mature thallus of *N. appendiculatum*.

usually diverge rather widely and develop almost at right angles to the main axis of the germ tube. The two branches soon begin to enlarge in the region of their origin and taper toward the extremities, and in this manner the spindle-shaped, medianly constricted apophysis is formed (fig. 2E). In the meantime its extremities continue to elongate and branch, until an extensive, richly branched rhizoidal system is established. As the rhizoids absorb nutriment from the host cell, the apophysis continues to enlarge (fig. 2G) and eventually reaches mature proportions.

The zoospore case and germ tube have persisted in the meantime, and the wall of the former soon begins to thicken. As this continues, it takes on a faint yellow tint which gradually changes to dark amber. As a result the wall of heavily infected *Nitella* and *Chara* cells frequently appears dotted with light brown globules, and by starting with these as points of observation, one can readily trace the germ tube down into the cell and locate the apophysis and rhizoidal system.

The latter becomes very extensive, as is shown in figure 1, branches freely, and at maturity may often cover a radius of 600 μ . It is usually variable in thickness and may attain a diameter of 5–6 μ , but its extremities eventually run out to fine filaments. Occasional spindle-shaped swellings which resemble the spindle organs of *Cladochytrium* may occur at irregular intervals (fig. 1A, 1B, 2I), but I have never so far observed them to become septate or give rise to secondary and tertiary, intercalary sporangia or resting spores. The thallus of *N. appendiculatum* thus appears to be monocentric as far as my observations go at present. Considered from the standpoint of phylogeny, the presence of intercalary swellings in the rhizoids may possibly foreshadow the evolution of the spindle and turbinate organs of the Cladochytriaceae and an initial stage in the transition to a polycentric mode of development.

As the rhizoidal system and apophysis mature in size, the absorbed nutriment accumulates to some extent in the latter, but this accumulation does not reach the same proportions as in apophysate species of *Chytridium* and *Phycochytrium*. In these species the greater part of the protoplasm of the zoosporangium is usually aggregated in the apophysis before the sporangium begins to develop, whereas in *N. appendiculatum* the latter usually begins to form before the remainder of the thallus is fully developed. The sporangium first appears as a protuberance on the surface of the apophysis and soon grows into a globular body (fig. 2G). It frequently develops on the upper or lateral surface close to the region of attachment of the germ tube, and in some cases it appears as if it had budded out from the germ tube itself, as is shown in figures 2H and 2K. The incipient sporangium continues to increase in size, and within 40–60 hours attains mature proportions. At maturity it is usually flattened, depressed, and predominantly kidney-shaped (fig. 2J–2N), although sub-spherical and elongated ones may also

occur and vary from $8 \times 14 \mu$ – $18 \times 30 \mu$ in their respective diameters.

In figure 2I is shown an early developmental stage in which the granular and somewhat refractive protoplasm is evenly distributed, while figure 2J shows a sporangium with two short tapering exit tubes which has attained mature size. As many as three tubes may occur on one sporangium and vary from blunt papillae to elongated necks, depending apparently to some degree on the position of the sporangium relative to the host wall. During maturation the sporangium becomes delimited from the apophysis and rhizoidal system by a cross wall (fig. 2K–2N). In the sporangium shown in figure 2J the refractive droplets are very minute and distributed in polygonal patterns, and as a result the protoplasm looks as if it has undergone cleavage. Whether or not this is true is impossible to determine with certainty in living material, but the subsequent changes in the refringent material suggests that cleavage does not occur until later.

A slightly later stage is shown in figure 2K, in which the minute refractive globules are apparently coalescing to form larger and more conspicuous bodies. Many of them are angular, constricted, somewhat irregular, and look as if they were fusing with adjacent ones. Coalescence continues until a more or less definite number of fairly equal globules are formed (fig. 1, 2L). As a result the protoplasm as a whole becomes less grey and granular in appearance, and the sporangium looks like a more or less hyaline vesicle in which are suspended a large number of pearly white refringent globules. The maturation of a fairly large number of sporangia has been followed at different time intervals, and the change from the finely granular stage (fig. 2J) to that shown in figure 2L takes place in approximately 6 to 8 hours.

The sporangia may remain in this condition for several hours, but eventually the tips of the exit tubes open. So far the initial stages of dehiscence have not been observed, and it is impossible to say whether or not the sporangia are operculate. The fully formed zoospores begin to ooze out, as is shown in figure 2M, and form a globular mass at the mouth of the exit tube. After lying quiescent thus for about a minute, they begin to glide upon each other, pull apart, and finally dart away (fig. 2N). The initial behavior of the zoospores is accordingly similar to that of *Chytridium*, *Endochytrium*, *Cladochytrium replicatum*, etc. In structure and general appearance they are strikingly similar to those of *D. intestina*, and except for a marked difference in the length of the cilium, it is impossible to differentiate them when they are together. As is shown in figure 2A, they are hyaline, spherical in shape when actively swimming, vary from 3.5–4.5 μ in diameter, with a 40 μ long posteriorly attached cilium, and possess a large clear refractive globule. Their method of swimming is typical of rhizidiaceous chytrids, darting about rapidly, coming to rest for a moment, and then 'dashing off again. They may occasionally become

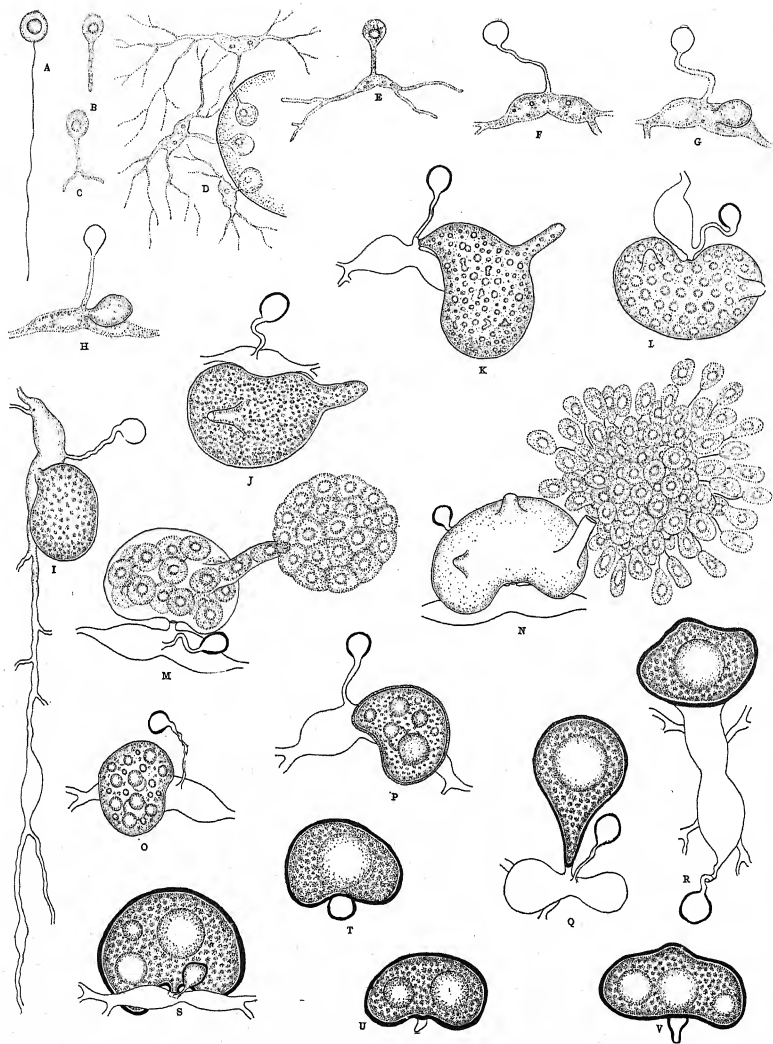


Fig. 2 showing the stages in the development of the thallus, zoosporangia, and resting spores.

amoeboid and creep around with the long cilium dragging behind.

DEVELOPMENT AND STRUCTURE OF THE RESTING SPORES.—The resting spores are similar to the sporangia in size and shape but dark amber in color with a thick wall and 1-4 large refractive globules. Figures 2A-2V show stages in resting spore development as well as their variations in size and shape. They are usually somewhat flattened, depressed, and kidney-shaped, and vary from $10 \times 18 \mu$ - $17 \times 26 \mu$. As far as my observations go at present, their method of development is fundamentally the same as that of the sporangia up to a certain stage, and at the start it is impossible to determine whether the young thallus is going to form a sporangium or resting spore. The latter buds out from the apophysis in the same fashion, but before it has reached any significant size, a visible change becomes apparent. A number of refractive globules appear in the cytoplasm and continually increase in number in the same manner as I (1936, 1937) have described for *Chytridium lagenaria*, *Phlyctochytrium chaetiferum*, and *Endochytrium operculatum*. By the time the incipient resting spore has reached mature proportions, they begin to coalesce into larger ones, as is shown in figure 2Q. Simultaneous with these internal changes the wall begins to thicken and takes on a light yellow tinge which deepens from light to dark amber as the spores mature (fig. 2P-2R). Further coalescence of the refractive globules occurs (fig. 2P) until usually only a large central one is present, as is shown in figures 2Q, 2R, and 2T. The apophysis, rhizoidal system, zoospore case and germ tube remain visible and attached for a long time, but as the host tissue disintegrates and rots away, they gradually become dissociated. So far no sexuality has been observed in the development of the resting spore, nor has germination been seen.

SYSTEMATIC POSITION AND RELATIONSHIP.—As far as it is known at present, *N. appendiculatum* belongs in the family Rhizidiaceae, but should it prove with further study to be polycentric, it would have to be

transferred to the Cladochytriaceae. Its apophysate sporangia and resting spores as well as its complete intramatrical development indicate some relationship with *D. intestina* perhaps, but in the development of the sporangium as an outgrowth of the apophysis it is more like *Phlyctochytrium stellatum*. According to Petersen (1909, 1910) and Scherffel (1926), however, the sporangium in the latter species may become partially extramatrical and apparently lacks exit tubes or papillae, while the resting spore is formed directly by the encystment of the entire globular apophysis. None the less, *P. stellatum* shows some tendency toward the *N. appendiculatum* type of development, and it is not improbable that when both species are completely known, they may possibly be included in the same genus.

SUMMARY

A new genus, *Nephrochytrium*, is tentatively proposed for an intramatrical, monocentric chytrid which is characterized by occasional intercalary swellings in its rhizoids and the development of zoosporangia and resting spores as outgrowths from the apophysis. Because of its monocentric thallus, intramatrical development, and apophysate sporangia and resting spores, it is included at present in the family Rhizidiaceae near *Diplophyctis*.

The type species, *N. appendiculatum*, occurs as a saprophyte in dead internodes of *Chara* and *Nitella* in association with *D. intestina* and other chytrids. Its sporangia are flattened, depressed, and usually somewhat kidney-shaped, $8 \times 14 \mu$ - $18 \times 30 \mu$, with 1-3 exit papillae or tubes of varying lengths. The resting spores are of much the same shape, $10 \times 18 \mu$ - $17 \times 26 \mu$, but light to dark amber in color with 1-4 large refractive globules. The zoospore case thickens, turns amber in color, and persists after germination on the surface of the host cell as an appendage to the sporangia and resting spores.

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NOTE ON SMITH'S PAPER ON STOMA NUMBER¹Horace W. Norton²

SMITH (1937) uses analysis of variance in the study of stoma number in *Phaseolus vulgaris*. It is hoped that some criticism of the statistics in this paper will help to clarify their uses and interpretation and to indicate some of the commoner errors in statistical work.

In Smith's table 1 an analysis of variance for each of six regions of the leaf is presented. There seems to be no reason for analyzing the regions separately, and the analysis could have been presented as in table 1 of this paper. (For interpretation of table 1 see Snedecor (1937), especially section 10.12.) Other points concerning Smith's table are (1) the eighth analysis does not add; (2) one fifth of the total sum of squares for the seventh analysis should be the sum of squares between pieces in the eighth analysis, but these figures are 75.7 and 74.6, respectively, a poor agreement; (3) adding the sum of squares within pieces for the six regions gives 168.8 and should coincide with the sum of squares within pieces in the eighth analysis, but the figure given is 179.0; (4) from the means of regions given in the text, the sum of squares between regions is 56.6, but 56.3 appears in the seventh analysis; (5) though the mean squares are given to two decimals, the second decimal is incorrect in many of them, as may be expected from the preponderance of ciphers in the second decimal; and (6) the last 5 per cent F should be approximately 1.52.

TABLE 1. Upper epidermis of 6G7-1-1.

Source of variation	d/f	Sum of squares	Mean square	F
Between regions ..	5	11.3	2.26	1.19
Between pieces				
within region	24	64.5	2.69	1.91 ^a
Within pieces	120	168.8	1.41	
Total	149	244.6		

^a Means significant at the 5 per cent level.

(The sums of squares between pieces within region, within pieces, and total may be in error by two or three in the first decimal since they were built up from values given by Smith.)

In discussing the lower epidermis, the 5 per cent and 1 per cent F values, 1.79 and 2.24, respectively, correspond to 12 and 300 degrees of freedom. It would seem that this analysis must include two leaves unless the number of regions is different for the lower than for the upper epidermis. It is not stated which of these alternatives is correct.

Though Smith's table 1 purports to be a complete analysis of the upper epidermis of 6G7-1-1, only 150 units were included; whereas it appears from table 2 that 300 units were counted. It is of interest to note that table 2 gives no indication of the number of units or "group," though Smith has sought to illustrate

the importance of that number in his discussion of data from the lower epidermis of 6G7-1-1. It may be seen from the accompanying text that the entry in table 2 for the lower epidermis of 6G7-1-1 is for 50 units per "group." As in the mean squares of table 1, there is a notable preponderance of ciphers in the terminal decimal place of the means given in table 2. From values given in the text, the mean for the upper epidermis of 6G7-1, 2, 3 and 4-3B is 8.73, but table 2 shows 8.70.

In discussing table 2, it is stated that "Comparable parts . . . are very uniform . . ." This statement seems inappropriate. The observed value of F, 1.20, with the 5 per cent F equal to 2.44, shows that the variation between leaves is not significantly greater than that within leaves. This condition is described as homogeneity, not uniformity. That is, the several leaves may be thought to have been drawn from one population rather than from two or more different populations, but not that the leaves themselves have the same mean value. If the variation between leaves were significantly less than that within leaves, it would seem appropriate to speak of uniformity.

Later in the discussion of table 2, it is said that because the observed F exceeded its 5 per cent value, further analysis of 6G7-1, 2, 3 and 4-3B is necessary. Since it is always possible to choose from a homogeneous population pairs of individuals which differ "significantly"—that is, are more than three standard deviations apart—it would seem that there is little to be gained by choosing, from a set of values which have been shown to be heterogeneous, pairs of individuals that differ significantly.

It is suggested that the analysis of variance may be strongly affected by discontinuity of distribution as in this case of counting number of stomata. The result of any count being necessarily an integer, continuity is lacking, and since Chi-square is much less sensitive to discontinuity, it might have been more appropriate. Also, since for Chi-square it is desirable that expected numbers be at least 5, preferably 10, a larger field should have been used.

It seems desirable that papers which use statistical techniques should be carefully reviewed and, if necessary, revised before publication. Further, if such papers are to be useful, they must give rather a detailed description of what data were collected. If the usual form of table for presenting results of the analysis of variance is used, this information is usually well enough set forth.

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¹ Received for publication October 19, 1937.

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NOTE ON SMITH'S PAPER ON STOMA NUMBER¹

Hugh B. Smith

THE CRITICISMS in the note by Norton of the use of the analysis of variance method by Smith (1937) are in general correct. Homogeneity was confused by Smith for uniformity, and the calculations were apparently carried to too few decimal places. Although 150 unit areas counted in the upper epidermis of the juvenile leaf were used to obtain the data of table 1, 300 unit areas were used to calculate the mean reported in table 2; 300 unit areas were also used to calculate the mean for the lower epidermis. 6G7-1-1 in table 2 should be changed to 6G7-1, 2, 3, and 4-1.

¹ Received for publication October 25, 1937.

Because of misinterpretation of analysis of variance, Smith is in error in regard to counts necessary for the determination of the mean number of stomata for the leaf as a whole. Counts should be made at random in all regions instead of in any region of the leaf.

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CONTRIBUTIONS TO THE CHEMISTRY OF THE PLANT CELL WALL.

VII. THE CELLULOSE IN THE RHIZOMES OF BRAKE FERN

(*PTERIS AQUILINA*)¹

William M. Harlow and Louis E. Wise

ALTHOUGH CELLULOSE is presumed by many workers to be the fundamental substance of plant cell walls (Wise, 1930), this assumption should not be made in any particular case without adequate chemical proof. The work of chemists has demonstrated the presence of repeated anhydro-cellobiose units in true cellulose. It may have been claimed that cellulose is present in the Pteridophytes on the basis of staining "reactions," but such tests seem to have little chemical significance (Harlow, 1928). No data have been found indicating a relationship between the cellulose of ferns, if present, and that of the higher plants or Spermatophytes.

From the chemist's viewpoint, the acetolysis reaction in which cellobiose octaacetate is derived from cellulose furnishes one of the best diagnostic tests for the latter's presence. Cellobiose-octaacetate is a definite substance which can be accurately identified by its crystal form (fig. 2, 3), melting point, and optical rotation. It has been prepared not only from cotton but also from wood cellulose (Wise and Russell, 1923) and more recently from that of Spanish moss (Meer and Wise, 1935).

SELECTION AND PREPARATION OF MATERIAL.—The rhizomes of brake fern (*Pteris aquilina* L.) were chosen for study because (1) this species is an important and widespread forest and range weed both here and abroad, and (2) ample material was available. An analysis of the structurally complex rhizome itself (fig. 1) was not undertaken, but attention was cen-

tered upon the fibrovascular strands which were readily stripped from the rhizome after the latter was pounded. The strands were washed in water with some rubbing between the fingers to remove a certain amount of starchy material from the ruptured parenchymatous cells surrounding the woody tissues, and were then oven-dried (105°C.). The dried material was brittle and easily broken into small pieces which were agitated on an 80-mesh screen in order to discard excessively powdered fragments.

METHODS AND PROCEDURE.—Unless otherwise stated, preliminary extractions, Cross and Bevan cellulose, and alpha cellulose were made according to the standardized procedures adopted by the Technical Association of the Pulp and Paper Industry (1932), while Spencer's technique (1929), using halved quantities of material and reagents, was used in the preparation of cellobiose-octaacetate.

The weighed oven-dried sample was extracted successively with an alcohol-benzol mixture, hot water, and finally alcohol. The alcohol-benzol and water extractives gave the solution a deep red color, while in the final extraction with alcohol, a yellow color was evident.

Cross and Bevan cellulose was prepared by successive chlorinations followed each time by treatment with sulfurous acid and hot dilute sodium sulfite. Although most of the material was white after the fifth chlorination, two more were used to complete the reaction upon some of the larger fragments which still had small, dark cores after sulfite treatment.

Since some difficulty was experienced in passing chlorine through the relatively large amount of pulp needed for subsequent experiments, another method

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The authors are indebted to Mr. Gordon MacCammon for help in collecting rhizomes of the fern and to Mr. William Davis for assistance in determining optical rotations.

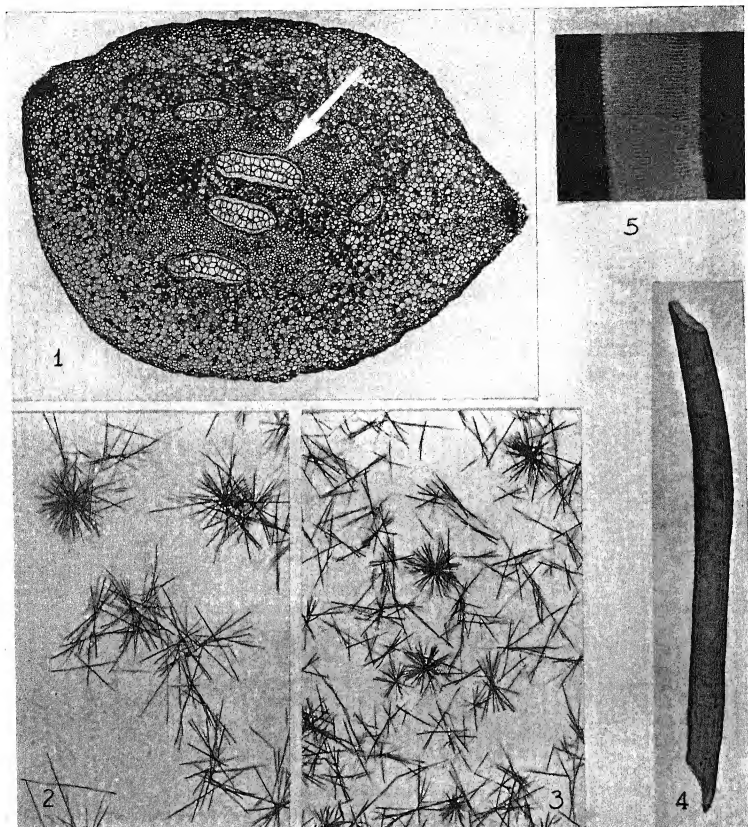


Fig. 1-5.—Fig. 1. Transverse section of brake fern rhizome (arrow shows one of the woody bundles). $\times 12$.—Fig. 2. Micro-crystals of cellobiose-octaacetate from brake fern cellulose.—Fig. 3. The same from cotton cellulose the difference in size is due merely to a difference in rate of crystallization).—Fig. 4. Vessel, or vessel-like segment, from brake fern wood. $\times 50$.—Fig. 5. A portion of the same, using polarized light and showing the linear its. $\times 150$.

cellulose preparation was tried using ethanol-nine² in the preliminary extraction of the oven-dried material. This solvent is alkaline, anhydrous, boils at 170°C . and not only frees the material from extractives but also removes the greater part of the gum. In one experiment, approximately 50 per cent of the oven-dried material was removed by a 5-hour extraction in a Soxhlet extractor over the boiling agent.

² Peterson and Wise, patent applied for, 1936.

The extracted material was placed in saturated chlorine water for one hour and then collected on a Gooch crucible, allowing the fibers to make their own mat. Subsequent treatment with sulfurous acid and hot sodium sulfite followed the standardized procedure. This bleaching process was repeated once, and finally the pulp was washed thoroughly with water, followed by alcohol and ether. The oven-dry weight compared favorably with that obtained by the much more laborious Cross and Bevan chlorination method, and the material was perhaps a trifle whiter.

Alpha cellulose was prepared from this material, using the prescribed strength of concentrated alkali.

One gram portions of this alpha cellulose and that prepared from cotton were subjected, respectively, to acetolysis under the conditions of time, temperature, and ratio of chemical reagents (H_2SO_4 to acetic anhydride) which Spencer (1929) found gave the maximum yields of cellobiose-octaacetate. The accompanying table summarizes the results obtained.

TABLE 1. Percentages of materials obtained from the woody strands of brake fern.

	% of oven-dry weight
Ash	2.2
Extractives (Ale-benzol, water, alcohol) ..	13.8
Cross & Bevan Cellulose (Chlorination) ..	29.4
Cellulose obtained by extraction with ethanolamine, followed by mild chlorination	30.0
Alpha cellulose from the above	17.0

TABLE 2. Cellobiose octaacetate from alpha cellulose of brake fern, and cotton.

	% of theoretical yield
Crude cellobiose-octaacetate from brake fern	43.4
Crude cellobiose-octaacetate from cotton	40.0
Crude cellobiose-octaacetate from cotton (acc. to Spencer)	42.3
Melting point of purified cellobiose-octaacetate from both brake fern, and cotton	225°C (uncorrected) ^a
Melting point of cellobiose-octaacetate from cotton (Spencer)	227.5°
Optical rotation (Specific)	
Cellobiose-octaacetate from brake fern	+ 43.5°
Cellobiose-octaacetate from cotton ..	+ 41.5°
Cellobiose-octaacetate from cotton (Spencer)	+ 41.4°

^a Cellobiose octaacetate from the two sources, respectively, was dissolved in chloroform to obtain optical rotation data. The octaacetate was recovered by evaporation of the solvent, and recrystallized from alcohol. That from cotton gave a melting point of 226°C, while in mixture [proportions $\frac{1}{4}$ to $\frac{3}{4}$] with that from brake fern, the same temperature was obtained.

DISCUSSION.—The fibro-vascular bundles in the ferns provide an interesting source of material for analysis, since they represent primary woody tissue not derived from a cambium, as is the case in many of the higher plants, and are also lacking in fibrous elements. As may be seen in figures 4 and 5, the wood consists mostly of scalariform-pitted vessels, or

vessel-like segments. An examination of the alpha cellulose showed a very few parenchymatous cells, perhaps about as many as might occur in similar material prepared from wood of the higher plants.

Although a comparative test with cotton was run, the moisture content of this sample was not definitely determined. Hence, for comparative purposes we have also cited Spencer's average yield of octaacetate. The higher figure for the octaacetate from brake fern is probably due to small amounts of impurities; this was indicated by a slight gray tinge of the crude acetate from the fern not observable in that prepared from cotton. After several recrystallizations from alcohol, this difference in color was not noticeable, and the purified material was then subjected to melting point tests and optical rotation. In the latter instance, readings can be considered only approximate due to the weakness of the solutions. Only about .5 gm. of substance was available for use, and the errors in determining the optical rotation are greatly magnified.

SUMMARY

Since cellobiose-octaacetate, a pure crystalline substance readily identified by its melting point, optical rotation, and crystal structure, can be prepared both from cotton and from the primary wood of brake fern, it may be assumed that the cellulose in the latter possesses the same repeated anhydrocellobiose unit as that found in the cellulose of the higher plants.

Although it may be strongly suspected that a similar condition obtains in the primary wood of other Pteridophytes, this cannot be accepted without a study of the wood of other species in this group.

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SOME CHROMOSOME NUMBERS IN THE CRUCIFERAE¹

Frank H. Smith

DURING AN investigation of various species showing prochromosomes, the author collected and examined material of several species of the Cruciferae. The chromosome numbers reported here were determined from this material. In so far as the author is aware, the numbers for species marked with an asterisk represent new counts. The other counts confirm earlier reports made by workers especially interested in the chromosome numbers in this family. A discussion of the many references to chromosome numbers in the Cruciferae is not attempted as it would be out of place in this article.

Entire flower buds of the various species were dipped in Carnoy's solution for approximately thirty seconds and then fixed in Navashin's solution or Karpechenko's modification of this solution. Paraffin sections were stained by the crystal violet-iodine-picric acid procedure.

**Arabis dentata* T. & G. (fig. 1) has six pairs of chromosomes which are relatively large for the Cru-

ciferae. This material was collected on limestone cliffs near Lake Winnebago, Wisconsin.

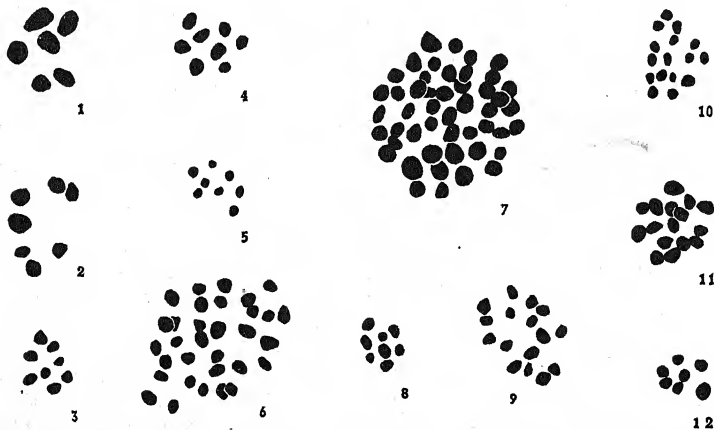
**A. laevigata* (Muhl.) Poir. (fig. 2) has seven pairs of chromosomes which are somewhat smaller than those of *A. dentata*. This species was collected in several localities in Wisconsin, and all material shows the same number of chromosomes.

**A. lyrata* L. (fig. 3), from Dewey Park, Wisconsin, has eight pairs of still smaller chromosomes.

Jaretsky (1928) has reported the chromosome numbers of nine known species of *Arabis*. Eight of these have eight pairs of chromosomes; *A. hirsuta* (L.) Scop. and an unknown species of *Arabis* both have sixteen pairs. The species formerly known as *A. hirsuta* in the United States has been recently distinguished from the Eurasian *A. hirsuta* by Hopkins (1937) and was designated *A. pycnocarpa*. The author collected material of **A. pycnocarpa* on Ferry Bluff, Wisconsin. While the fixation was very poor, it was determined with a fair degree of certainty that sixteen pairs of chromosomes are present. These species are probably tetraploid forms, but the numbers for other species reported here indicate that eight is not necessarily the basic number for this genus.

¹ Received for publication November 24, 1937.

The author is especially grateful to Dr. Milton Hopkins, Department of Botany, University of Oklahoma, who made the taxonomic determinations of the species reported here.



All figures were drawn with the aid of a camera lucida at a magnification of 4300 diameters and have not been reduced in publication. Figure 4 shows the chromosomes on the plate during the homoecotypic division; all other figures show them during equatorial-plate stages of the heterotypic division.

Fig. 1-12.—Fig. 1. *Arabis dentata*.—Fig. 2. *Arabis laevigata*.—Fig. 3. *Arabis lyrata*.—Fig. 4. *Barbarea vulgaris*.—Fig. 5. *Cardamine parviflora*.—Fig. 6. *Cardamine pennsylvanica*.—Fig. 7. *Draba arabisma*.—Fig. 8. *Erysimum auranthoides*.—Fig. 9. *Lepidium apetalum*.—Fig. 10. *Lepidium virginicum*.—Fig. 11. *Roripa armoracia*.—Fig. 12. *symsbrium altissimum*.

Barbarea vulgaris R. Br. (fig. 4), collected near Sun Prairie, Wisconsin, has eight pairs of chromosomes. This count coincides with those made by Manton (1932) in this and other species of the same genus.

**Cardamine parviflora* L. (fig. 5) has eight pairs of rather small chromosomes; **C. pennsylvanica* Muhl. (fig. 6) has thirty-two pairs of somewhat larger chromosomes. These species were collected near Devil's Lake, Wisconsin. These counts agree with those made by Manton (1932) in other species of *Cardamine*. Most of the species in this genus show eight pairs of chromosomes, but polyploid species with 32, 48, and 64 chromosomes occur.

**Draba arabisans* Michx. (fig. 7), collected near Oakfield, Wisconsin, has forty-eight pairs of chromosomes. Eight is probably the basic number for this genus, but, curiously enough, the majority of the species reported thus far (Heilborn, 1927) are polyploids of this number. *D. arabisans* is the only species in this genus in which as many as forty-eight pairs of chromosomes have been observed. An additional study of the American species of this genus should prove interesting.

Erysimum cheiranthoides L. (fig. 8) has eight pairs of chromosomes. This material also came from the cliffs near Lake Winnebago. The same number of chromosomes was observed by Jaretsky (1928) in this species.

**Lepidium apetalum* Willd. (fig. 9) and *L. virginicum* L. (fig. 10) both have sixteen pairs of chromosomes. These counts correlate with those of eight pairs found by Jaretsky (1929) and of eight and sixteen by Manton (1932) in other species of *Lepidium*.

**Roripa armoracia* (L.) Hitchc. (fig. 11), collected near Fox Lake, Wisconsin, has sixteen pairs of chromosomes. Manton reported in this same genus two species with sixteen (8 pairs) and one with thirty-two (16 pairs) chromosomes.

Sisymbrium altissimum L. (fig. 12), collected near Sun Prairie, Wisconsin, has seven pairs of chromosomes. This number agrees with the earlier one reported by Manton.

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ORIGIN OF THE SEED COATS IN MAGNOLIA¹

T. T. Earle

INCIDENT to a study of the embryology of *Magnolia grandiflora* L., the manner of development of the seed coats from the integuments was noted. This does not agree with the development of the seed coats of *M. macrophylla* Michx., as described by Brandza (1891), whose findings have been accepted by some recent workers, but is identical with the development of the seed coats of *M. Umbrella* Desr. [*M. tripetala* L.], as described by Gray in 1858. While it is desirable that a careful re-examination of *M. macrophylla* be made, it seems reasonable to assume that the manner of the development of the seed coats in the several species of *Magnolia* does not differ in any important detail.

The outer integument of the ovule of *M. grandiflora* at the time of the opening of the flower-bud is made up of about ten layers of parenchymatous cells (fig. 1, o), while, except in the micropylar region, the inner integument consists of only three layers of cells (fig. 1, i). The cells of the outer layer of the inner integument are compressed and flattened against the middle layer, and their nuclei are long and spindle-shaped. The cells of the inner two layers are approximately isodiametric and have nearly spherical nuclei,

and those of the innermost layer stain very heavily with haematoxylin. These three layers can be easily identified at all later stages of development and are very valuable in correlating the parts of the mature seed. That part of the inner integument that surrounds the micropyle is usually four or five cells in thickness and forms a sort of plug beneath the micropylar opening in the outer integument.

The outer integument of the ovule develops into a testa of five distinct zones (fig. 3, o). A two- or three-layered epidermis makes up the external covering of the seed. Under this there is a zone made up of two or three layers of flattened cells. Beneath this there is a zone of fleshy tissue made up of three to six layers of large, thin-walled parenchymatous cells, some of which contain much volatile oil. According to Evans (1933), the fleshy coat contains about 57 per cent oil and a quantity of reducing sugars. Beneath this zone of large cells there is a zone consisting usually of two layers of smaller cells which separates the outer fleshy tissue from the stony tissue beneath. This stony tissue is about four layers of cells in thickness except in the region immediately under the vascular bundle of the raphe, where it forms a ridge that projects into the endosperm and

¹ Received for publication December 6, 1937.

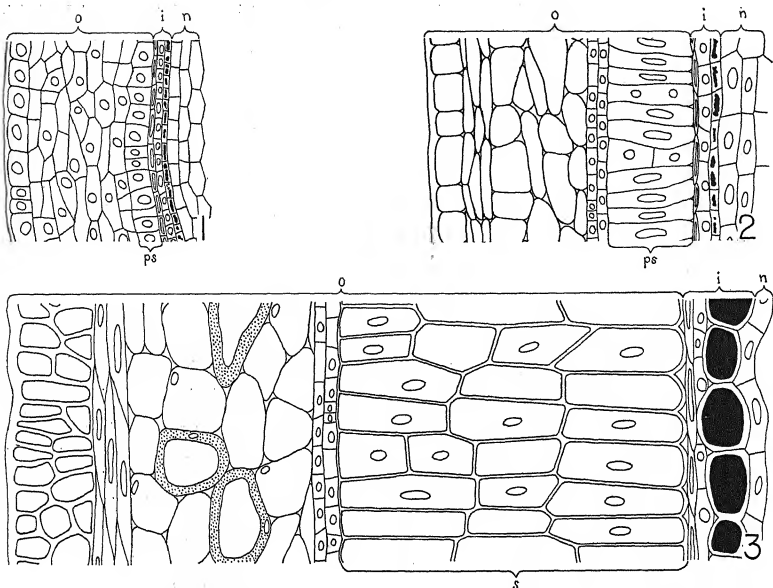


Fig. 1-3.—Fig. 1. Portion of integuments and nucellus of ovule at time of opening of flower-bud.—Fig. 2. An intermediate stage.—Fig. 3. Corresponding section in a nearly mature seed. The stippled cells are oil cells. *o*, outer integument; *i*, inner integument; *n*, nucellus; *ps*, primordium of stony tissue; *s*, stony tissue.

is about twelve to fifteen cells thick. The cells in this zone are usually very regular in shape and have comparatively thick, lignified walls. Gray described this zone in *M. tripetala* as being derived from the inner part of the outer integument, as it certainly is in *M. grandiflora*, but Brandza maintained that in *M. macrophylla* the stony tissue is developed from the inner integument of the ovule.

The tegmen, which Brandza described (in *M. macrophylla*) as nucellar remains, is unmistakably a derivative of the inner integument of the ovule (fig. 3, *i*). It is thin and membranous and consists of three layers of cells: an outer layer consisting of long, compressed cells with elongated nuclei; a middle layer made up of approximately isodiametric cells with spherical nuclei; and an inner layer made up of relatively larger cells which contain a dark brown substance that practically obscures the spherical nuclei. An extremely thin sheet of nucellus remains

attached to the tegmen (fig. 3, *n*). The only other part of the nucellus that is not resorbed by the endosperm is a small mass at the micropylar end of the seed on which the embryo rests. That part of the inner integument that originally formed the greater part of the micropyle becomes greatly compressed by the stony tissue of the mature seed.

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"ROOT-PRESSURE"—AN UNAPPRECIATED FORCE IN SAP MOVEMENT¹

Philip R. White

THE LONG and tortuous history of our knowledge—or better our observations and theories—on the movement of sap in plants is familiar to most botanists. From the time of Stephen Hales, 200 years ago, up to the middle of the 19th century, the movement of water through the plant was supposed to be brought about primarily through the activity of living cells, either by the agency of unidentified tissues of root and stem, as Hales believed (1727), or the medullary rays, as suggested by Knight (1801). The "drawing power" of the leaves was recognized by both Hales and Knight, but, since it was well known that a suction pump would not lift water higher than about 30 feet, this was considered of secondary importance. Beginning about 1860, along with the rise of mechanistic theories in other fields, evidence began to accumulate which indicated that living cells might not be necessary for the rise of sap. This changing viewpoint found strong support in Molisch's demonstration (1902) of the traumatic nature of many of the bleeding phenomena upon which Hales' vital theory rested and finally culminated in the development of the Dixon-Askenazy cohesion theory of sap movement (Dixon and Joly, 1895; Askenazy, 1895). This theory, which takes account of the enormous suctions developed at evaporating leaf surfaces and of the fact that in capillary tubes water possesses a great tensile strength capable of transmitting these suctions through a plant stem to the soil, seemed to deal with forces more nearly commensurate with the needs of tall trees than had those demonstrated by Hales and his successors. The result was that most plant physiologists completely abandoned the vital theories in favor of the mechanical ones. In spite of the objections of Priestley (1935), Ursprung (1906), Heyl (1933), and others, that is approximately where the situation rests today.

The cohesion theory certainly has some very serious flaws which are rather well outlined in Priestley's paper of two years ago (1935). It has been accepted not so much because of its freedom from objections as because of the inadequacy of all other theories. The only real contender—Hales' old "root-pressure" scheme—was rejected, first because pressures greater than the 1.4 atmospheres recorded by Hales himself had not been observed, and second because all demonstrations of root-pressure rested on experiments with decapitated and moribund plants subject to Molisch's criticism. Water must be raised in some Eucalyptus trees to a height of 350 feet, requiring either a push or a pull of at least 13 atmospheres. The maximum root-pressures observed were only of a magnitude of

1.4 atmospheres, they were transitory, and doubt even existed as to their presence in uninjured plants. Consequently, modern plant physiology texts for the most part treat root-pressure as unimportant.

Excised roots of tomato have been kept growing in vitro for a number of years (White, 1937). The roots form normally developed vascular strands, although completely immersed in a nutrient solution (fig. 1). The occurrence of strands in roots grown under these conditions has seemed rather anomalous. If, however, a continuous flow of liquid be assumed to take place through the roots, the presence of strands would be understandable. If such a current exists, it was thought these roots might offer a means

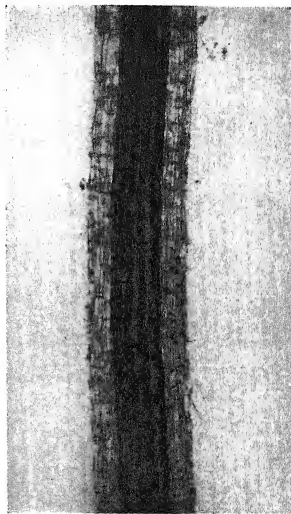


Fig. 1. Root of tomato (*Solanum lycopersicum* L.) showing the well developed vascular strand. $\times 90$. (Photograph by J. A. Carlie.)

of determining the reality or unreality of "root-pressure" and perhaps of measuring it under conditions free from Molisch's objections.

Means have, therefore, been devised of repeating Hales' original experiment, using single actively metabolizing tomato roots instead of his moribund grapevine stocks.

Capillary manometers were built to receive single roots. Specimens of a clone of excised tomato roots grown in continuous culture for the past 5 years were

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subcultured and allowed to stand for one week, to give the cut surfaces time to heal completely. Their bases were then carefully inserted into manometers, and seals were made by means of miniature rubber hose corresponding to those used in the classic *Fuchsia* demonstration known to every student of plant physiology. The roots with their attached manometers were returned to fresh flasks of nutrient, and their subsequent behavior observed (fig. 2). All manipulations had, of course, to be carried out aseptically and with as little trauma as possible.

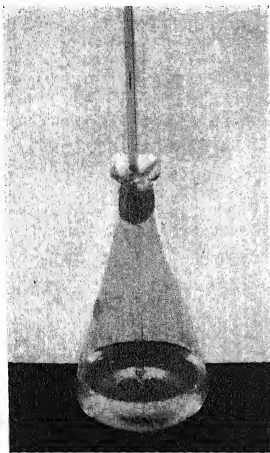


Fig. 2. Tomato root with attached manometer. (Photograph by J. A. Carlile.)

The results of the first experiments were surprisingly good (White, 1936). The roots did secrete water from their bases into manometers. There does exist a unidirectional flow of liquid through these roots. Root-pressure is not an artifact but a reality. This paper proposes to present some quantitative results of these experiments.

If roots of this kind are set up in two series of manometers, with capillaries of the same diameter, one series containing water, the other mercury, we would expect a decrease in the secretion rate under the mercury column corresponding to the 13.5:1 differential in weight between mercury and water. Figure 3 shows the result of such an experiment using manometers 500 mm. high. Contrary to expectation, no such observable decrease occurred. Both columns rose at the same rate. The pressure differential apparently had no effect on the secretion rates. The only conclusion to be drawn is that the curves do not represent pressures at all but only volumes.

Closed manometers in which the pressure would build up rapidly with very little volume change were tried, but such manometers proved hard to clean and did not permit the detection of leaks at the root-manometer juncture. Leveling bulbs were then resorted to. Results of an experiment with such a device are represented in figure 4. During the course of the experiment it was thrice necessary to replace or extend the manometer tube, and since the tubes were not all of the same diameter, a different scale had to be used for each. Scales were chosen, such that the slope of the curve for an hour or so before and after each change should be approximately the same. In this experiment the water column rose 1790 mm. in 6 days. A leveling bulb was then attached, and an atmosphere of pressure—760 mm. Hg—was applied. Since the column continued to rise at an undiminished rate, the pressure was increased

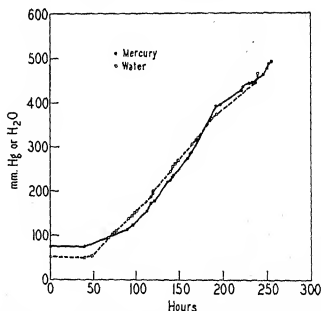


Fig. 3. Curves showing rise of liquid in two similar manometers, one filled with water, the other with mercury. The roots failed to secrete during the first 48 hours after the experiment was set up, presumably until they had recovered from the shock of manipulation. This lag does not always occur. The curves have almost identical slopes in spite of the 13.5:1 differential in weight between mercury and water.

after 20 hours to 2 atm. (1520 mm. Hg). The water still continued to rise. Sufficient mercury to give another atmosphere of pressure would have carried the bulb through the ceiling of the room, so the apparatus was carefully moved to a nearby laboratory which had a stairway in it, and the bulb was carried into the room above. Unfortunately, a period of adjustment was not allowed, as should have been done, so that when, under 3 atmospheres pressure, the column began to fall, no definite cause could be assigned for the drop. But the fact that when the pressure was reduced again to 2 atm., the column did not resume the upward course it had previously held at that pressure, indicated that a leak in the apparatus had probably been brought about in moving. The important fact demonstrated by this experiment is that a pressure of 2 atm. was not sufficient

to noticeably retard secretion of water from the uninjured base of a single root of tomato.

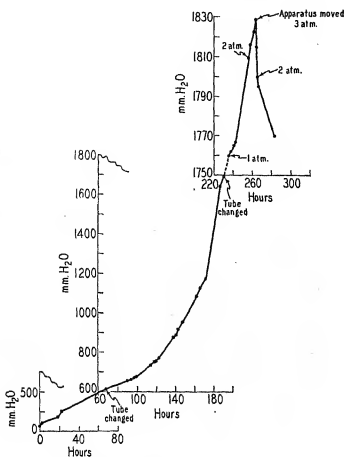


Fig. 4. A curve showing the effect of imposing pressures up to 3 atm. against the secretion pressures developed by a single tomato root.

Since roots of this sort developed pressures in excess of 30 pounds to the square inch, it was evident that something more than the simple hose seal between glass and root would have to be devised to stand the pressures. After many trials, the assembly shown in figure 5 was developed. The base of the manometer had a hole about 1 mm. in diameter and 15 mm. deep to receive the base of the root. Above this the opening tapered abruptly to a bore of about 0.25 mm. A centimeter back of the tip on the outside was a collar of glass. The specially made rubber connecting tube had a corresponding collar inside, with the hole about 1.5 mm. in diameter at the end which was to fit over the glass and only about 0.1 mm. in diameter where it was to enclose the root. In setting up an experiment, this tube was moistened with glycerine and the end of the manometer inserted completely through it so that the tip protruded. The base of the root was then inserted into the manometer and the rubber pushed back until it enclosed the root for a distance of about a centimeter and the glass for an equal distance. A strong linen thread was bound tightly around the rubber both over the root and over the glass. The rubber distributed the pressure so that this binding did not crush the root, while the glass collar prevented the rubber from slipping off. The whole was then enclosed in a metal clamp which had collars to press into the rubber at both ends. This effectively protected the rubber from being ruptured by the pressures applied. The

manometer was then inserted into a 500 ml. Erlenmeyer flask and the upper end attached to a metal manifold. Pressure was applied to the manifold from a compressed air tank and observation begun. The manifold used took 4 manometers at a time.

In the first series of experiments with this apparatus, a gauge reading to 100 pounds per square inch was used, on the supposition that this would suffice to record any pressure obtained. Figure 6 represents the results of one experiment with such a setup. The rise of the water column was observed for 24 hours and the secretion curve, which showed a definite diurnal variation in slope,² plotted. Pressure of one

² This diurnal rhythm was observed in all experiments where readings were made at frequent enough intervals to permit its detection. It seems to be a regular characteristic of the secretion process. The roots used were not protected against the diurnal variations in temperature (24°-28°C., June, 1936) and illumination characteristic of a laboratory room with N. E. exposure. Nevertheless, since the mean daily temperatures often varied more than did the hourly temperatures within single 24-hour periods, without producing corresponding variations in secretion rate, it seems improbable that this rhythm is the result of temperature variations. It is difficult, though not impossible, to imagine how an organ without chlorophyll and whose growth rate has been shown to be independent of illumination of the intensity obtained in the laboratory (White, 1937) could have this one process so markedly affected by illumination. This diurnal rhythm remains an interesting but as yet unexplained feature of the secretion process.



Fig. 5. Assembly, consisting of glass manometer, rubber connecting tube, and metal clamp, by which a single tomato root can be attached to a recording apparatus and its secretion force measured. (Photograph by J. A. Carlile.)

atmosphere was applied at 4 p.m. and, since the column continued to rise, a second atmosphere was applied at 5 p.m. and the apparatus left over night. Under a pressure of 2 atm. the column rose as rapidly that night as it had in the corresponding period of the day before, so at 9 a.m. the pressure was increased to 3 atm., at 11 a.m. to 4 atm., at 2 p.m. to 5 atm., and at 4 p.m. to 6 atm. At 7 o'clock that evening the column was still rising, and by 9 the next morning had risen an additional 110 mm., although it had been subjected to a pressure of 90 pounds per square inch over that period. As already stated, the gauge read only to 100 lbs. Another atmosphere would have given a pressure of 105 lbs., so the attempt to reach a pressure sufficient to stop secretion had to be abandoned. The pressure was, therefore, removed and the remainder of the curve to the top of the manometer plotted. A control curve, obtained without imposed pressure, is plotted above the experimental one, for comparison.

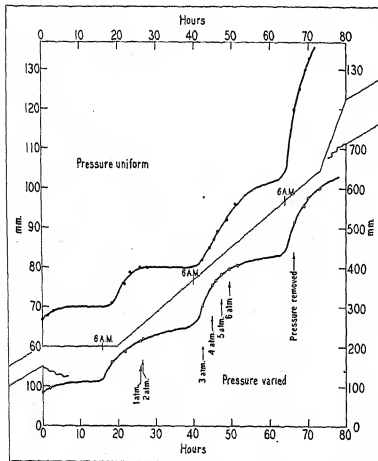


Fig. 6. Curves showing the rates of secretion of two similar tomato roots over a period of 3½ days, one with uniform (atmospheric) pressure, the other against imposed pressures up to 90 lbs./sq. in.

If, now, the control curve, recorded without imposed pressure, is compared with the experimental one, obtained under pressures varying between 0 and 6 atm., they are seen to be almost exactly alike. Moreover, if the segment: 9 a.m. to noon, on the 3rd day, is compared with the corresponding segment on the 4th day, their slopes are seen to be almost identical, although the second was obtained under zero pressure and the first under 3 atm. This record

represents one of 4 manometers set up in series on a single manifold. A second gave an identical curve up to the afternoon of the 3rd day when a bacterial contamination of the culture solution set in and secretion stopped abruptly. A third gave a similar curve but with a shallower slope, possibly because the root used had a smaller diameter, and the fourth developed a leak around the root which permitted air to escape. Six atmospheres pressure, 4 times the greatest value that I have found recorded elsewhere for root-pressure (the exudation pressures of Boehm, Figdor, Moliseh, and MacDougal were of quite a different sort, in that they were obtained as a result of trauma) was not enough to slow down secretion to a measurable extent.

The sap movement which we are here studying is a matter of filtration through a membrane which we usually assume to be readily permeable to water, and surely equally permeable in either direction. The membrane can, therefore, be ignored in any calculation of the forces causing filtration, at least so long as movement is slow. We have been trying to balance a measured applied force against an unknown secretion force. Secretion should stop when the external and internal forces are equal, and water should flow back through the membrane—the root—when the applied force exceeds the force of secretion. Secretion should become slower and slower as the external force approaches the value of the internal one.

Six atmospheres external pressure did not bring about any observable retardation of secretion from these roots. Only one conclusion appears possible from this observation—6 atmospheres must be so small in comparison with the secretion pressure actually developed by the roots as to be quite insignificant. It is the writer's opinion that this secretion pressure can not be less than 10 atmospheres and is probably much more than that. In fact, it seems possible that it may be limited only by the osmotic value of the cells themselves. Attempts have been made to impose still higher pressures, but they have met with mechanical difficulties that have not yet been overcome. It is interesting that so far failures have all been due to flaws in the apparatus. The roots have not failed to secrete liquid regularly against all pressures to which they have been subjected.

The old "root-pressure" theory of Hales has been disparaged by modern plant physiology texts because it did not provide sufficient force and because of the suspicion that it might be an artifact. These experiments seem to show conclusively that both objections are invalid. "Root-pressure" is certainly a very real phenomenon, going on in uninjured normally metabolizing roots and showing a striking diurnal rhythm that is reminiscent of many vital processes. The fact that a bacterial contamination will stop secretion so suddenly indicates the metabolic character of the process. And the force developed is of a magnitude not to be despised. Six atmospheres pressure is sufficient to sustain a 200 ft. water column. This is far higher than any tomato plant ever grows.

Yet such a column appears to be small in comparison with what the lifting power of tomato roots is capable of sustaining. That is a force which is to be reckoned with. It has been unappreciated in the past because, before the development of the root-culture technique, only moribund and abnormally metabolizing tissues could be studied.

It is not suggested that mechanical factors, such as transpiration pull, cohesion, capillarity, etc., do not play a considerable role in sap movement. Under conditions of high transpiration they probably do account for the movement of large volumes of water through the plant. It is merely pointed out that under certain conditions—such as those prevailing in the spring when the maple sap is flowing, though leaves have not been developed—some or all of these mechanical processes cease to function. At such times root-pressure or its equivalent, stem-pressure, is quite adequate to provide for the proper functioning of even the tallest trees.

No attempt will be made at present to explain how this force is developed. It may be said, however, that we are much interested in determining if there is a diurnal rhythm in respiration rate to correspond

with the observed rhythm in rate of secretion. It will take some rather delicate methods to determine that. But it is believed that we have here a phenomenon which can be studied in detail and which may throw some light on the mechanism of secretion.

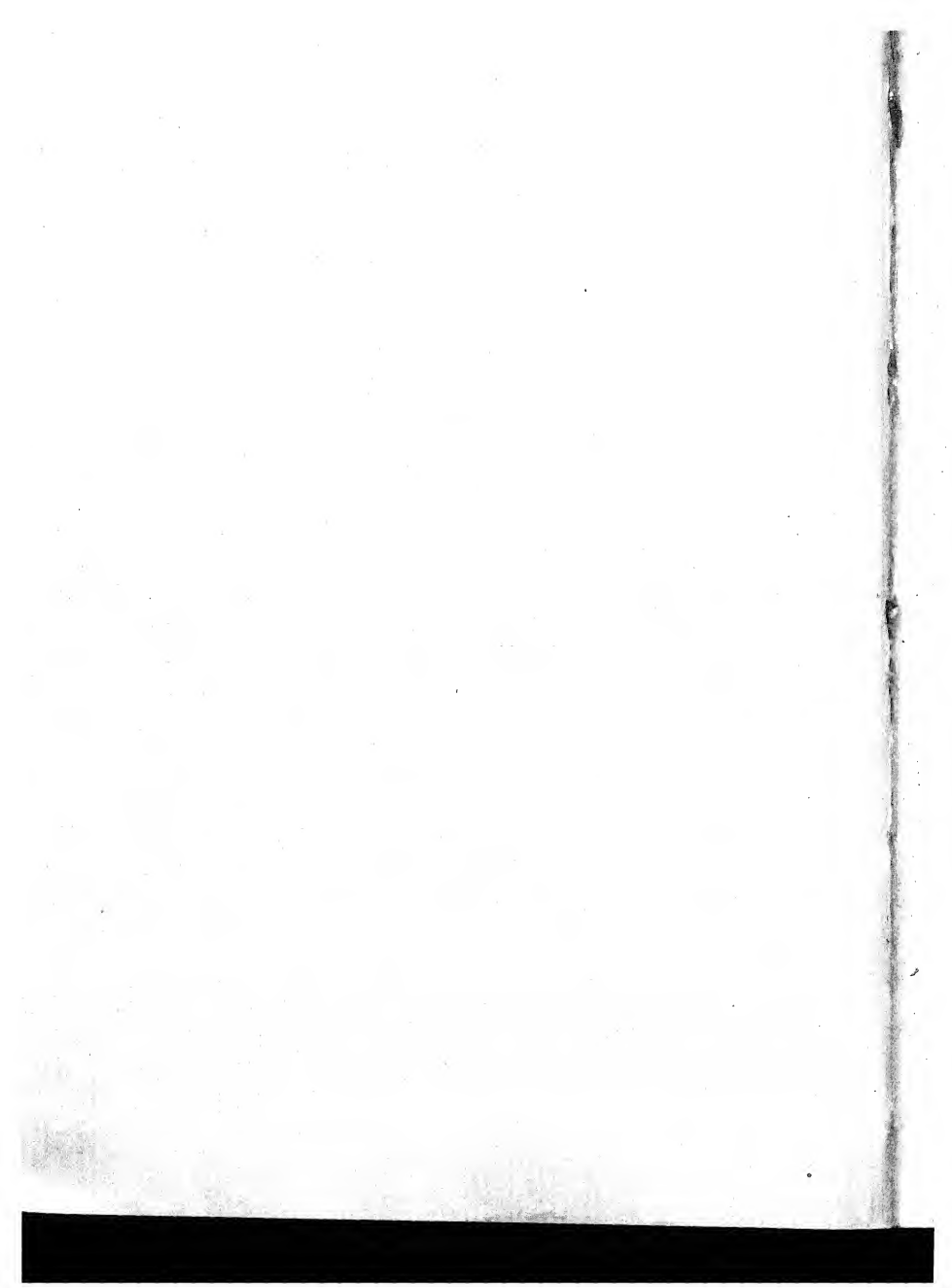
SUMMARY

Experiments have shown that excised tomato roots growing *in vitro* secrete sap continuously and rhythmically from their proximal ends. Methods of measuring the force of this secretion have been developed. It is not retarded by opposed pressures of 90 lbs. per sq. in. The secretion force, therefore, probably greatly exceeds this value. Since a pressure of 90 lbs. per sq. in. is sufficient to raise water to a height of 200 feet, and since the existence of such secretion pressures has been demonstrated in normally metabolizing, actively growing roots, it is concluded that "root-pressure" may be a far more important factor in sap movement than has been generally conceded.

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VITAMIN B₁ OR ITS INTERMEDIATES AND GROWTH OF CERTAIN FUNGI¹

William J. Robbins and Frederick Kavanagh

SCHOPFER (1934a, 1934b) reported that *Phycomyces Blakesleeanus* Burgeff does not grow upon a medium of mineral salts, pure dextrose, asparagine, and water, but grows normally on the same medium to which a minute quantity of crystalline vitamin B₁ is added. Schopfer's discovery resulted from a suggestion made to him by Burgeff (Schopfer, 1934c) based on the latter's observation of the effect of crystalline vitamin B₁ on the growth of *Phycomyces*. Other fungi found by Schopfer (1935) to require vitamin B₁ include *Absidia ramosa*, *Parasitella simplex*, *Mucor Ramannianus*, *Dicranophora fulva*, *Chaetocladium Brefeldii*, *Choanephora cucurbita*, *Choanephora persicaria*, and *Pilaria anomala*. Earlier reports on the importance of vitamin B₁ as a growth factor for the lower plants have been published by various investigators including R. J. Williams (1920, 1930), Lepeschkin (1924), and others. The conclusive evidence of the importance of vitamin B₁ (aneurin or thiamin) for the growth of plants depended upon a supply of the pure vitamin, which became available in 1934.

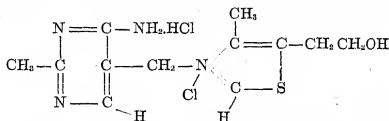
We have confirmed Schopfer's report of the effect of vitamin B₁ on the growth of *Phycomyces Blakesleeanus*, and since there are many organisms which do not grow, or grow poorly, on the common synthetic media (those lacking some natural organic supplement), we have determined the effect of vitamin B₁ on a number of fungi.

MATERIALS AND METHODS.—In addition to *Phycomyces Blakesleeanus*, a plus strain kindly furnished by A. F. Blakeslee, we have used other organisms secured through the courtesy of W. E. Maneval, J. T. Middleton, or C. M. Tucker. Those furnished by Maneval were *Absidia glauca* Hagem, *Basidiobolus ranarum* Eidam, *Mortierella* sp. no. 11, *Mortierella candelabrum* v. Tiegham and le Monnier, *Mucor circinelloides* v. Tiegham, *Mucor griseo-lilacinus* Poval, *Mucor* sp. no. 103, *Mucor* sp. no. 115, *Circinella aspera* Leadner, *Cunninghamella* sp. no. 102, *Thamnidium elegans* Link, *Zygorhynchus Moelleri* Vuill, *Zygorhynchus* sp. no. 111, *Rhizopus nigricans* Ehrenberg, + and —, *Phycomyces nitens* (Agardh) Kunze and Schmidt, +, *Sphaerulina trifolii* Rostr. From Tucker we secured cultures of *Phytophthora Boehmeriae* Saw., *P. cactorum* (L. and C.) Schwet, *P. cambivora* (Petri) Buis, *P. capsici* Leon., *P. cinnamoni* Rands, *P. colocasiae* Raeb., *P. cryptogea* Pethyb. and Laff., *P. Drechsleri* Tuck., *P. palmivora* Butler, *P. parasitica* Dast., *P. jagopyri* Takimoto; *Agaricus campestris* Linn., *Schizophyllum commune* Fr., *Sclerotium delphinii* Welch, and *S. Rolfii* Sac. Middleton furnished cultures of *Pythium arrhenomanes* Drechsler, *P. polycladon* Sideris, *P.*

scleroteichum Drechsler, *P. hyphalosticton* Sideris, and *P. Butleri* Sybramanian.

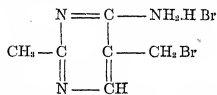
The fungi were grown in 125 cc. Erlenmeyer flasks containing 20 or 25 cc. of liquid medium. Two basic media were used. Medium B consisted of MgSO₄·7H₂O, 5.0 g.; KH₂PO₄, 15.0 g.; asparagine, 10.0 g.; dextrose (Cerelease), 100.0 g.; mineral supplements,² 1 cc.; redist. water 1000 cc. Medium C was medium B plus 0.5 g. of NH₄NO₃ per liter and 5.0 g. of asparagine and 50 g. of dextrose per liter instead of 10 g. and 100 g., respectively. Both media had a pH of 4.3. Crystalline vitamin B₁ (Betabion) (or its intermediates) from Merck and Company was added to the medium before sterilization at 12 lbs. pressure for from 15 to 20 minutes. The fungi were grown at room temperature ranging from 20° to 26°C.

Vitamin B₁ has the following structural formula:

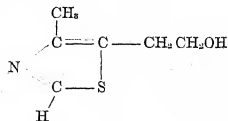


The two intermediates used were as follows:

2 methyl-5-bromomethyl-6-aminopyrimidine hydrobromide.



4-methyl-5β-hydroxyethylthiazole



These intermediates were used by Williams and Cline (1936) in synthesizing vitamin B₁, and for brevity are referred to in this paper by the terms pyrimidine and thiazole. The compounds were used were supplied through the courtesy of Merck and Company.

² The mineral supplements were contained in a modification of Hoagland's A to Z mixture prepared by adding to 18 liters of water LiCl, 0.5 g.; CuSO₄·5H₂O, 1.0 g.; FeSO₄, 1.0 g.; H₃BO₃, 1.0 g.; Al₂(SO₄)₃·18H₂O, 1.0 g.; SnCl₂·2H₂O, 0.5 g.; MnSO₄·4H₂O, 7.0 g.; NiCl₂·6H₂O, 1.0 g.; Co(NO₃)₂, 1.0 g.; TiSO₄, 1.8 g.; KI, 0.5 g.; NaBr, 0.5 g.

¹ Received for publication December 11, 1937.

[The JOURNAL for March (25: 141-227) was issued March 21, 1938.]
AMERICAN JOURNAL OF BOTANY, VOL. 25, NO. 4, APRIL, 1938

EXPERIMENTAL RESULTS.—*Phycomyces Blakesleeanus* made almost no growth in a medium to which no vitamin B₁ had been added. The effect of the addition of vitamin B₁ is illustrated by the following experiment in which various amounts of the vitamin were added to 20 cc. of medium B contained in 125 cc. Erlenmeyer flasks. The solutions were inoculated with two drops of a spore suspension in sterile distilled water. At the end of three weeks the flasks were heated to kill the spores and mycelium, the mycelium was removed, dried at 94°C. and weighed (table 1). Under the conditions of this experiment the growth in the absence of vitamin B₁ was inappreciable. The addition of 0.01 unit^a (0.00346 γ) of the vitamin per flask increased the dry weight of mycelium produced. This solution contained one part of the vitamin in about 6,000,000,000 parts of solution. However, this quantity and 0.1 unit did not permit the development of aerial mycelium and sporangia. With 0.3 unit a sparse aerial mycelium developed and some sporangia were formed. With increasing amounts of the vitamin the growth was increased, though not proportionately. This is shown by the ratio (table 1) between the dry weight of the mycelium formed and the quantity of vitamin furnished. Some factor other than the quantity of vitamin B₁ evidently limited growth at the higher concentration of the vitamin. The results are shown graphically in the photograph, figure 1, taken at the end of twelve days' growth.

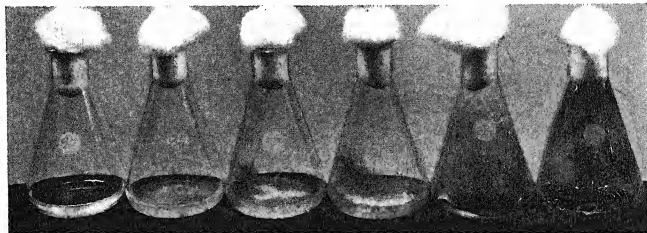


Fig. 1. Effect of vitamin B₁ on growth of *Phycomyces Blakesleeanus* in solution B. From left to right quantity of vitamin B₁ per flask (20 cc. solution) 0, 0.1 unit, 0.3 unit, 1 unit, 3 units, 10 units. One unit = 0.346 γ .

The other fungi listed were grown in 25 cc. of medium C and in the same solution to which 30 units (10.38 γ) of vitamin B₁ had been added to each flask. The experiment was performed in triplicate.

The following organisms were found to grow satisfactorily in medium C and to be unaffected by the addition of the vitamin so far as macroscopic observation of their development indicated: *Agaricus campestris*, *Absidia glauca*, *Basidiobotus ranarum*, *Circinella aspera*, *Cunninghamella* sp. no. 102, *Mortierella candelabrum*, *M.* sp. no. 11, *Mucor circinnelloides*, *M. griseobolacinus*, *M.* sp. no. 103, *M.* sp. no. 115,

^a A unit is 10⁻⁹ mole of the compound.

Table 1. Effect of vitamin B₁ on growth of *Phycomyces Blakesleeanus*. Dry weights are average of triplicate cultures grown for three weeks in 20 cc. of medium B containing mineral salts, asparagine, dextrose, and the amounts of B₁ indicated.

Amount of vitamin B ₁ per flask (10 ⁻⁹ mole)	Dry weight of mycelium (mgm.)	Ratio weight mycelium to weight of vitamin B ₁
0.00	0.3	—
0.01	2.0	500,000
0.10	18.0	500,000
0.30	38.0	360,000
1.00	83.0	240,000
3.00	233.0	220,000
10.00	500.0	140,000
100.00	580.0	17,000

Thamnidium elegans, *Zygorhynchus Moelleri*, Z. sp. no. 113.

The original cultures used for inoculation were grown on potato dextrose agar. Care was used in inoculating the culture solutions to avoid transferring any of the agar medium or touching the agar with the transfer needle. Nevertheless, we considered the possibility that sufficient vitamin B₁ was contained in the spores or in the mycelium used as inoculum to permit growth in the medium lacking vitamin B₁.

Transfers of each of these organisms were, therefore, made from the growth in medium C to the same medium. No difference was noted in the growth of any of the above fungi in their second period of growth in the medium lacking the vitamin. While dry weights were not determined and only the transfers on a medium lacking the vitamin were made, it appears probable that these fungi need no external supply of vitamin B₁, or other accessory growth factor, in order to grow normally in the medium used.

The following fungi, however, grew poorly or not at all in solution C, but very well in the same solution to which the vitamin had been added: *Phyto-*

phthora Boehmeriae, *P. cactorum*, *P. cambivora*, *P. capsici*, *P. cinnamomi*, *P. cryptogea*, *P. Drechsleri*, *P. palmivora*, *P. parasitica*, *Schizophyllum commune*, *Sclerotium delphinii*, *S. Rolfsii*, *Pythium polycladon*, *P. arrhenomanes*, *Sphaerulina trifolii*, and *Phycomyces nitens*.

A bit of mycelium from a culture on potato dextrose agar or corn meal agar was used for inoculating the liquid in the flasks for all the fungi in the second group (those favored by vitamin B₁) except for *Phycomyces nitens*. The spores of this organism were used for inoculation.

Phycomyces nitens, a plus strain originally secured from Cornell University, responded as did *Phycomyces Blakesleeanus*. In the solution lacking B₁, a very small amount of submersed mycelium developed from the spores of the original inoculation. In the presence of the vitamin, the surface of the liquid was covered with a heavy mycelial mat, and the flask was filled with aerial sporangioophores.

Sclerotium delphinii, *S. Rolfsii*, *Schizophyllum commune*, and *Sphaerulina trifolii* grew poorly in the solution lacking the vitamin; all the mycelium was submersed. Subcultures of the best growth in the solutions lacking vitamin B₁ were made to the same medium. *Sphaerulina trifolii* failed to grow, *Schizophyllum commune* grew somewhat but not as much as in the original culture. Both *Sclerotium delphinii* and *S. Rolfsii* made some growth in the subcultures, the former developing submersed colonies about 1 cm. in diameter and the latter colonies about 2 cm. in diameter. In the medium used, *Schizophyllum commune* and *Sphaerulina trifolii* did not grow in the absence of vitamin B₁, and *Sclerotium delphinii* and *S. Rolfsii* grew slowly through two transfers. In the presence of vitamin B₁, entirely different results were secured. Within a few days the mycelium of *Sclerotium delphinii* and *S. Rolfsii* had covered the liquid in the flasks, and at the end of two weeks both organisms had developed a heavy mycelial mat and numerous typical sclerotia (fig. 6). *Sphaerulina trifolii* and *Schizophyllum commune* also grew well in the presence of vitamin B₁ (fig. 2).

With the exception of *Phytophthora Boehmeriae* and *P. cambivora*, none of the *Phytophthoras* grew appreciably in the solution lacking vitamin B₁. Subcultures of these two *Phytophthoras* made from the small amount of growth in the cultures lacking vitamin B₁ into the same medium failed to grow. In the presence of the vitamin, however, the nine species of *Phytophthora* mentioned grew well. At the end of 17 days the following notes were made of the growth in the solution containing vitamin B₁: *P. Drechsleri*—mycelium in single mass, all submersed, with radiating hyphae reaching to edges of flask; *P. cryptogea*—much like *P. Drechsleri*; *P. Boehmeriae*—very heavy growth, gelatinous-like mycelium all submersed and nearly filling liquid in flask; *P. capsici*—much like *P. Boehmeriae* but growth not so heavy; *P. cambivora*—mycelium all submersed, growth like *P. Drechsleri* but heavier; *P. parasitica*—some powdery

aerial mycelium, somewhat heavier than *P. Drechsleri*; *P. cactorum*—much like *P. parasitica*; *P. cinnamomi*—very heavy growth, liquid in flask filled with mycelium, surface covered with powdery aerial mycelium; *P. palmivora*—mycelium all submersed and in isolated clumps.

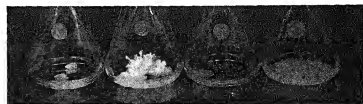


Fig. 2. Effect of vitamin B₁ on growth of *Schizophyllum commune* (left) and *Sphaerulina trifolii* (right) in solution C. From left to right: no B₁, 30 units B₁, no B₁, 30 units B₁.

Eventually all nine species filled the liquid in the flasks with mycelium. Without vitamin B₁, the species of *Phytophthora* named above failed to grow in the medium used, but with the addition of the vitamin, the growth was good (fig. 3).

Pythium arrhenomanes (fig. 3) and *P. polycladon* responded, as did the *Phytophthoras* used. Without the vitamin little or no growth occurred, while the addition of the vitamin to the medium resulted in good growth.

The response of *Rhizopus nigricans*, + and — strains, differed from that of both groups of fungi previously discussed. This fungus grew well in medium C. In fact, we have carried it through four subcultures in this medium without evident change in growth. However, instead of being unaffected by the addition of the vitamin, the growth of this organism was inhibited (fig. 4). The growth of the minus strain was inhibited more than that of the plus strain. Schopfer (1935) has reported the inhibitory action of vitamin B₁ on the growth of *Rhizopus nigricans*.

The fungi with which we have worked may be divided into four groups: (1) Those which grew satisfactorily in the medium used and were not markedly affected by the addition of vitamin B₁. (2) Those which grew poorly in the medium used and were markedly favored by the addition of vitamin B₁. (3) Those which did not grow in the medium used but grew well in the same medium to which vitamin B₁ had been added. (4) Those which grew in the medium used and were inhibited by the addition of vitamin B₁.

Phytophthora fagopyri, *Pythium scleroiteichum*, *P. hyphalosticton*, and *P. Butleri* failed to grow uniformly in medium C either without vitamin B₁ or with vitamin B₁. *Phytophthora colocasiae* grew poorly in medium C and in the same medium plus the vitamin. We are uncertain whether the failure of these organisms to develop was due to unsatisfactory material used in the inoculation, to the unfavorable character of the basic medium (hydriion concentration, solute concentration), or to lack of growth substances other than vitamin B₁. From evi-



Fig. 3. Effect of vitamin B₁ on growth of fungi in solution C. 1, *Phytophthora cambivora*; 2, *P. cactorum*; 3, *P. cinnamomi*; 4, *P. Boehmeriae*; 5, *P. cryptogea*; 6, *P. capsici*; 7, *P. palmivora*; 8, *P. Drechsleri*; 9, *P. parasitica*; 10, *Pythium arrhenomanes*. The left flask for each fungus contains 30 units vitamin B₁.

dence presented later in this paper, we believe that *Phytophthora fagopyri* and *Pythium Butleri* also respond to vitamin B₁, while *Pythium sclerotiechum* does not.

In the experiments reported above, the vitamin was added to the culture solution before sterilization. High temperatures decompose vitamin B₁ (Williams, Waterman, and Gurin, 1929), and we have noted an odor of thiazole in an autoclaved distilled water solution of 100 units of vitamin B₁ per cc. It is probable, therefore, that in our culture solutions an unknown proportion of the vitamin had been decomposed into its thiazole and pyrimidine intermediates. We (Robbins and Kavanagh, 1937) have demonstrated that *Phycomyces Blakesleeanus* grows normally if supplied with both thiazole and pyrimidine instead of vitamin B₁, and that pyrimidine and thia-

zole (Robbins, Bartley, Hogan, and Richardson, 1937) cure polyneuritis in pigeons. We have found also (Robbins and Bartley, 1937) that excised tomato roots grow in a solution of mineral salts, sugar, and thiazole. Will the intermediates of vitamin B₁ replace the vitamin molecule for the fungi discussed above?

Phycomyces nitens in our experiments required both intermediates, as does *Phycomyces Blakesleeanus*. The thiazole or pyrimidine alone was ineffective. A mixture of thiazole and pyrimidine, or the pyrimidine alone, was effective for the growth of *Phytophthora fagopyri*, *Sclerotium delphinii*, *S. Rolfii*, *Sphaerulina trifolii*, *Pythium polycladon*, and *P. Butleri*. The thiazole alone was ineffective. *Pythium sclerotiechum* grew well with thiazole or pyrimidine alone and in the mixture of the two intermediates.

The eight organisms mentioned above were grown in solution C with sufficient Na₂HPO₄ added to make the reaction pH 5.2. Twenty-five cc. of this solution were placed in 125 cc. Erlenmeyer flasks of Pyrex glass. To some 30 units (4.3 γ) of thiazole were added, to others 30 units (8.5 γ) of pyrimidine, and to still others a mixture of 30 units of thiazole and 30 units of pyrimidine. Each series was run in triplicate.

and sclerotia were somewhat slower in developing. With the thiazole alone the mycelium was submersed, much smaller in amount, and no sclerotia developed. The growth of *Sphaerulina trifolii* was much like that of the two species of *Sclerotium*, though less heavy.

Phytophthora jagopyri, *Pythium polycladon*, and *P. Butleri* grew little in the solution containing thiazole alone but filled the liquid in the flask with mycelium in those solutions containing pyrimidine or



Fig. 4. *Rhizopus nigricans*,—strain, grown in solution C. Three flasks to left, 30 units vitamin B₁; three flasks to right, no vitamin B₁.

At the end of two weeks the growth of *Phycomyces nitens* in the solution containing both thiazole and pyrimidine was heavy, almost filling the flask with aerial mycelium and sporangioophores, while little or no growth developed with one intermediate alone (fig. 5).

Sclerotium delphinii and *S. Rolfii* had formed a heavy mat covering the liquid in the flasks containing both pyrimidine and thiazole and had formed numerous sclerotia (fig. 6). In the solutions containing pyrimidine, a heavy mat had formed, which, however, was not so heavy as with both intermediates,

the mixture of thiazole and pyrimidine. *Pythium scleroteichum* grew well in the liquid containing thiazole, pyrimidine, and the mixture. These results are summarized in table 2.

Do *Phytophthora jagopyri*, *Pythium Butleri*, *P. polycladon*, *Sclerotium delphinii*, *S. Rolfii*, and *Sphaerulina trifolii* require pyrimidine only, or do they require both intermediates, synthesizing under the conditions of our experiments sufficient thiazole but insufficient pyrimidine for normal growth? To determine whether or not these organisms form thiazole in the culture solutions which contained pyrimidine only, we proceeded as follows:

At the end of two weeks' growth the cultures were sterilized by autoclaving and the mycelium removed. The sterile culture solutions which contained the metabolic products of the various fungi were inoculated with the spores of *Phycomyces Blakesleeae*. This fungus requires an external supply of both intermediates for growth. At the end of eleven days observations were made on the growth of *Phycomyces*. The growth of the *Phycomyces* was least (see table 3) in the cultures originally containing thiazole, greater in the cultures originally containing pyrimidine, and greatest (except for *Pythium Butleri*) in the cultures originally containing the mixtures of thiazole and pyrimidine.

The growth of *Phycomyces* (table 3) in solutions in which *Phytophthora jagopyri*, *Pythium polycladon*, and *Sphaerulina trifolii* had grown indicates that the latter fungi had formed a small amount of pyrimidine in the thiazole solutions and some thiazole in the

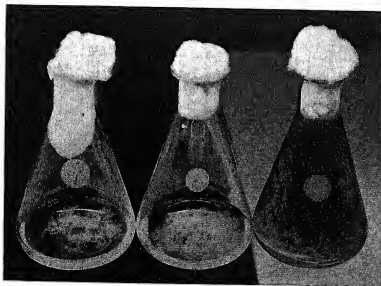


Fig. 5. *Phycomyces nitens* grown 15 days in solution C of pH 5.2. From left to right; 1, plus 30 units thiazole; 2, plus 30 units pyrimidine; 3, plus 30 units thiazole and 30 units pyrimidine.

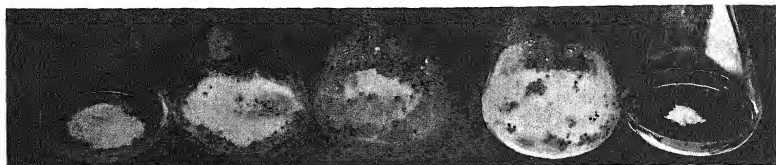


Fig. 6. *Sclerotium Rolfsii* grown 15 days in solution C. From left to right: 1, plus 30 units thiazole; 2, plus 30 units of pyrimidine; 3, plus 30 units thiazole and pyrimidine; 4, plus 30 units vitamin B₁; 5, no supplement. 1, 2, and 3 in solution C of pH 5.2; 4 and 5 in solution C pH 4.3.

pyrimidine solutions, though not enough thiazole for good growth of *Phycomyces*. *Pythium Butleri*, however, formed sufficient thiazole in the pyrimidine solution to permit good growth of *Phycomyces*. *Pythium scleroteichum* synthesized more pyrimidine in the thiazole solutions than the other fungi. It formed more thiazole in the pyrimidine solution than *Phytophthora jagopyri*, *Pythium polycladon*, or *Sphaerulina trifolii* but less than *P. Butleri*.

Comparing the results summarized in table 2 and table 3, we can say that *Pythium Butleri* (fig. 7) synthesized in the thiazole solution insufficient pyrimidine for good growth of *Phycomyces* and insufficient for its own good growth; in the pyrimidine solution it synthesized sufficient thiazole for good

have confirmed the effect of vitamin B₁ on the growth of *Phycomyces Blakesleanus* and have found similar results for *Phytophthora cactorum*, *Helvella infula*, *Polyporus adustus*, *P. abietinus*, *Fomes pinicola*, and *Trametes serialis*. They found vitamin B₁ to exert a favorable effect also upon the growth of *Nectrina coccinea* and *Sclerotinia cineria*. Leonian (1936a, 1936b) concluded that vitamin B₁ was not the effective agent in the natural products which were necessary for the growth of *Phytophthora cactorum*. This conclusion followed from his observations showing that treatments of the natural products which would destroy vitamin B₁ as such did not destroy the favorable action of the natural product upon the growth of *P. cactorum*. Nevertheless, our results and

TABLE 2. Relative growth of the fungi named in solution C of pH 5.2 plus thiazole, pyrimidine, or a mixture of the two intermediates.

Organisms	Thiazole	Pyrimidine	Thiazole and pyrimidine
<i>Phytophthora jagopyri</i>	Light	Very heavy	Very heavy
<i>Sclerotium delphinii</i>	Light	Heavy	Very heavy
<i>Sclerotium Rolfsii</i>	Light	Heavy	Very heavy
<i>Sphaerulina trifolii</i>	Light	Medium	Heavy
<i>Pythium polycladon</i>	Light	Heavy	Heavy
<i>Pythium Butleri</i>	Almost none	Heavy	Heavy
<i>Pythium scleroteichum</i>	Heavy	Heavy	Heavy
<i>Phycomyces nitens</i>	Almost none	Almost none	Heavy

growth of *Phycomyces*. The amount of thiazole formed in the pyrimidine solution was adequate also for good growth of the *Pythium*. *Phytophthora jagopyri* (fig. 8) synthesized in the thiazole solution insufficient pyrimidine for good growth of *Phycomyces* and for its own good growth also; in the pyrimidine solution it synthesized insufficient thiazole for good growth of *Phycomyces*. Enough thiazole, however, was formed in the pyrimidine solution for good growth of the *Phytophthora*.

DISCUSSION.—Good growth of a number of parasitic fungi was secured in our experiments by the addition of vitamin B₁ to a medium of mineral salts, asparagine, and dextrose in which little or no growth occurred without the vitamin. Kögl and Fries (1937)

those of Kögl and Fries show that the addition of vitamin B₁ to a medium in which *P. cactorum* does not grow permitted good growth of this organism. We have found, however, that *Phycomyces Blakesleanus* and *P. nitens* respond to a mixture of thiazole and pyrimidine intermediates of vitamin B₁, and for six of the fungi with which we have worked the pyrimidine alone added to a basic nutrient medium was sufficient for growth. It seems possible that the presence and activity of the intermediates of vitamin B₁ may account for Leonian's results.

In previous papers we have pointed out that *Phycomyces Blakesleanus* requires an external supply of both intermediates for normal growth; the thiazole alone or the pyrimidine alone is ineffective.

Sclerotium delphinii, *S. Rolfsii*, *Phytophthora jagopyri*, *Pythium polycladon*, *Pythium Butleri*, and *Sphaerulina trifolii* require an external supply of pyrimidine, the thiazole alone is ineffective or relatively so.

Are both thiazole and pyrimidine needed by a fungus, *Pythium Butleri* for example, which grows well

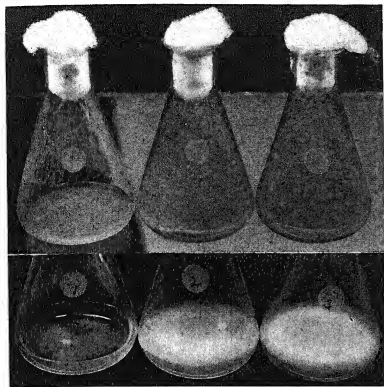


Fig. 7. Below, *Pythium Butleri* grown in solution C of pH 5.2. From left to right: 1, plus 30 units thiazole; 2, plus 30 units pyrimidine; 3, plus 30 units each intermediate. Above, same cultures as below sterilized, mycelium removed, and inoculated with *Phycomyces Blakesleeanae*.

with pyrimidine alone in the medium but poorly with thiazole alone? It is our opinion that both intermediates are needed. The evidence presented above showing that *Pythium Butleri* formed thiazole in a solution which originally contained pyrimidine alone and in which it grew well supports the assumption that both intermediates are needed by these fungi for growth. *Phycomyces Blakesleeanae* synthesizes neither; *Pythium Butleri*, under the conditions of our experiments, synthesizes enough thiazole for good growth but insufficient pyrimidine. *Absidia glauca* (and numerous other fungi) synthesizes sufficient of both for its normal development.

Are the intermediates effective individually or is the vitamin B₁ molecule as such the effective agent? We have presented evidence that the vitamin B₁ molecule is probably the effective agent for *Phycomyces Blakesleeanae* (Robbins and Kavanagh, 1937). There is no specific support for this view in the work reported here. On the other hand, our findings that organisms which grow well with an external supply

of pyrimidine alone synthesize thiazole and do not grow with thiazole alone would be in accord with the assumption that the vitamin B₁ molecule is the effective agent.

SUMMARY

Burgeff's and Schopfer's discovery of the importance of vitamin B₁ for the growth of *Phycomyces Blakesleeanae* was confirmed.

A number of saprophytic fungi grew satisfactorily for two transfers in a liquid medium of mineral salts, asparagine, and dextrose and were unaffected by the addition of 30 units per flask of vitamin B₁. (A unit is 0.346 γ or 10^{-9} mole.)

A number of parasitic fungi grew poorly, or not at all, in a liquid medium of mineral salts, asparagine, and dextrose but grew very well in the same medium to which 30 units of vitamin B₁ was added to each flask.

Rhizopus nigricans, + and —, grew satisfactorily in a liquid medium of mineral salts, asparagine, and dextrose, but its growth was materially reduced by the addition per flask of 30 units of vitamin B₁; the growth of the minus strain was more affected than that of the plus strain.

Phycomyces nitens grew well in a medium of mineral salts, asparagine, and dextrose to which 30 units per flask of the thiazole and pyrimidine intermediates were added. The addition of thiazole alone or of pyrimidine alone was ineffective.

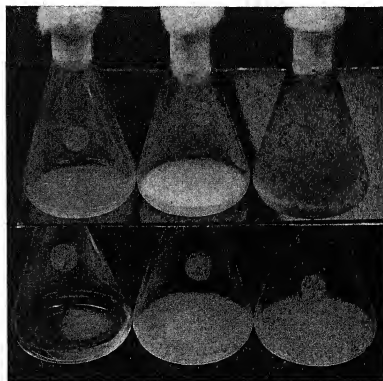


Fig. 8. Below, *Phytophthora jagopyri* grown in solution C of pH 5.2. From left to right: 1, plus 30 units thiazole; 2, plus 30 units pyrimidine; 3, plus 30 units each intermediate. Above, same cultures sterilized, mycelium removed, and inoculated with *Phycomyces Blakesleeanae*.

TABLE 3. Relative growth of *Phycomyces Blakesleeanus* in solution C of pH 5.2 containing thiazole only, pyrimidine only, or a mixture of thiazole and pyrimidine in which the various fungi named had already grown. Compare with table 2.

	Thiazole	Pyrimidine	Thiazole and pyrimidine
<i>Phytophthora fagopyri</i>	+	+(+)	++++
<i>Pythium Butleri</i>	+	++++	++++
<i>Pythium polygladon</i>	+	-(+)	++++
<i>Pythium sclerotieichum</i>	++	++(+)	++++
<i>Sphaerulina trifolii</i>	+	+(+)	++++

+ = small amount submersed mycelium.

+(+) = heavier than +.

++ = aerial mycelium 1 to 3 cm. high, few or no sporangia.

++(+) = heavier than ++.

++++ = good development of aerial mycelium with sporangia.

Phytophthora fagopyri, *Pythium Butleri*, *P. polygladon*, *Sclerotium delphinii*, *S. Rolfsii*, and *Sphaerulina trifolii* grew well in a medium of mineral salts, asparagine, and dextrose to which 30 units per flask of thiazole and pyrimidine intermediates were added, or to which 30 units of pyrimidine alone were added. The addition of thiazole alone was ineffective.

The synthesis of thiazole by some of the fungi

when grown in a pyrimidine medium was demonstrated.

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FURTHER STUDIES ON ARTIFICIAL PARTHENOCAРРY¹

Felix G. Gustafson

IN THIS study on induced parthenocarpy the writer has had two objects in mind: to test out new compounds and to obtain further information on the mechanism of fruit development. The following new compounds have been used: 9,10-di-n-propyl-9,10-dihydroxy-9,10-dihydro-1,2,5,6-dibenzanthracene and its potassium salt; 9-phenanthrylacetic acid and its potassium salt; theelin (oestrone); methylcholanthrene; Skatol; crude oestrin from pregnancy urine; 1,2,5,6-dibenzanthracene; N-methyl-pyrrole-2,5-dipropionic acid; pyrrole- α -acetic acid; pyrrole- α -carboxylic acid; and the potassium indole acetate.

These compounds were chosen for various reasons. Some were chosen because they have shown oestrogenic properties, others because of carcinogenic activities, others because they have been reported to cause growth in plants, while still others were chosen because it was thought there might be a correlation between molecular structure and effectiveness in stimulating fruit development. The first four compounds were synthesized by Prof. Bachmann, and the pyrrole compounds were made by Mr. Safir, under the direction of Prof. Blicke, all of the Chemistry Department at the University of Michigan. The potassium indole acetate was made from indole acetic acid bought from Eastman Kodak Company. At the time the potassium indole acetate was made and used in experiments with tobacco, there was no published record of it having been used as a growth hormone, but since then Avery, Burkholder, and Creighton (1937) and also Zimmermann and Hitchcock (1937) have published on its growth activity. It should, however, be remembered that salts have previously been supplied to plants in buffered growth substance solutions, and in 1935 Thimann used the sodium salts of indole-, indene-, and cumaryl-acetic acids. The pyrrole compounds have not been used heretofore as growth-promoting substances. Their synthesis is exceedingly difficult.

In examining the structure of the compounds that have been found to be active, it occurred to the writer that the pyrrole group might be the active radicle in the indole compounds. Pyrrole acetic, -propionic, and -butyric acids were not available

commercially, and the question arose as to how to obtain them. On inquiry it developed that Prof. Blicke had synthesized pyrrole, and he consented to supervise the synthesis of compounds containing the pyrrole ring. Unsuccessful attempts were made to synthesize pyrrole mono-propionic acid, and the best that could be done was the synthesis of N-methyl-pyrrole-2, 5-dipropionic acid. Pyrrole- α -acetic acid was very difficult to make, and only after several attempts was success attained. The beta compound has so far been unattainable.

During the winter greenhouse-grown plants were used, while in the summer the plants were grown in the field. The chemical was applied as a lanolin paste as in previous experiments (Gustafson, 1936) or injected into the ovary according to the method used by Yasuda (1934). Controls were run with and without pure lanolin or injection with distilled water.

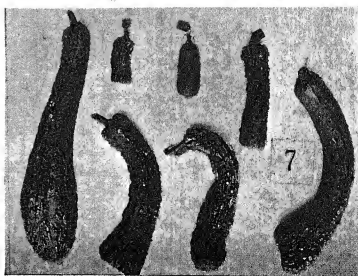
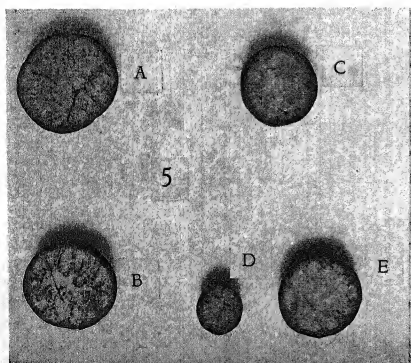
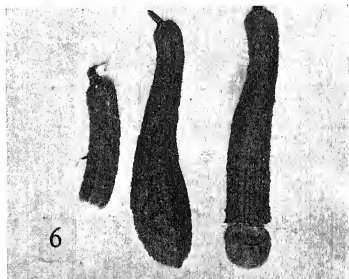
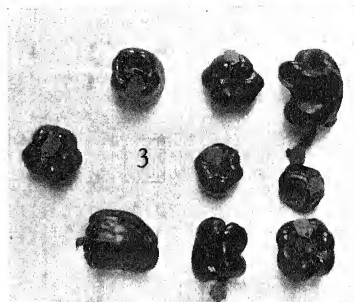
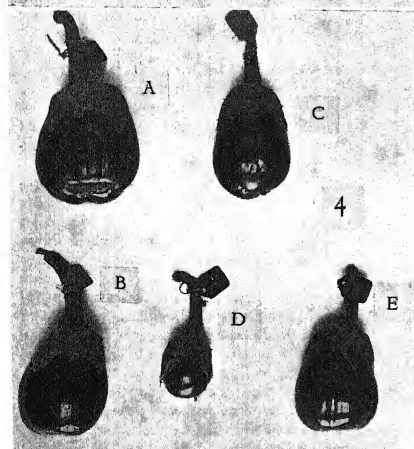
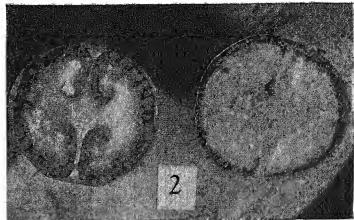
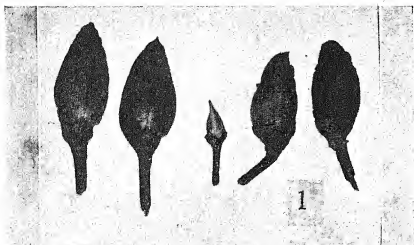
RESULTS.—Seven of these compounds used in concentrations designated within the brackets following the name were without any influence on the ovaries: theelin (0.6 per cent in lanolin); methyl-cholanthrene (5 per cent in lanolin); 1,2,5,6-dibenzanthracene (5 per cent in lanolin); N-methyl-pyrrole-2,5-dipropionic acid (5 per cent in lanolin); 9,10-di-n-propyl-9,10-dihydroxy-9,10-dihydro-1,2,5,6-dibenzanthracene (1 per cent in lanolin) and its potassium salt (0.2 per cent aqueous solution); and the potassium salt of 9-phenanthracene (about 0.1 per cent in lanolin). Oestrin, Skatol, and 9-phenanthrylacetic acid caused some growth of the ovaries of several flowers. Oestrin produced fruits in *Salpiglossus* that were from $\frac{1}{8}$ to $\frac{1}{4}$ normal size; 9-phenanthrylacetic acid (0.2 per cent aqueous solution) caused some development in *Godezia* and *Clarkia*; while Skatol (0.5 per cent in lanolin) caused a very slight but unmistakable growth in *Matthiola*. All the compounds used in concentrations of one or less per cent are very insoluble, and if higher concentrations had been used, the compounds would not have gone into solution.

Of the thirteen new compounds used only three were definitely successful: the Pyrrole- α -carboxylic, pyrrole- α -acetic acids, and the Potassium indole acetate. The last named was by far the best, and, while no quantitative experiments have been conducted, the potassium salt of indole acetic acid seems to be as effective as the acid itself, if not more so.

Potassium indole acetate.—The K-indole acetate was the first of the three compounds to be used. In the greenhouse it was used on *Clarkia elegans*, *Salpiglossus variabilis*, *Godezia* sp., snapdragon, tomato, Maryland mammoth variety of tobacco, and cucumber. In the field, apple, pepper and eggplant flowers were treated with this compound. The K-indole

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acetate was used either as a 5 per cent paste in lanolin or as a 0.2 per cent aqueous solution and injected. In all but the apple it produced an enlargement of the ovary.

Clarkia, *Salpiglossus*, *Godetia*, and snapdragon were treated only with the paste. The fruits produced on these plants were from one-half to normal size. The tobacco was both injected and treated with the paste. The latter produced considerable growth but never a normal sized or shaped fruit. The injection into the ovary was made through the pedicel with an ordinary syringe, using the finest obtainable needle. The needle was inserted into the pedicel, pushed into the ovary, and then withdrawn slightly before pressure was applied to the plunger. This procedure prevented clogging of the needle. Injection was continued until the air spaces in the sepals were filled with liquid, a condition which was easily observable. Sometimes the needle was accidentally pushed clear through the ovary, but when it was withdrawn, the ovary and the sepals were nevertheless completely injected.

Figures 1 and 2 picture the results obtained with the tobacco. On the left are the fruits obtained by pollination, in the middle of figure 1 is the ovary, and on the right of both figures are the fruits produced by injecting the ovary with K-indole acetate. The artificially produced fruits were usually somewhat smaller, and the tip was more often bent and somewhat shrivelled, showing signs of less complete development than the fruits produced by pollination. The ovules grew considerably and looked somewhat like small seeds, but from the large number that were placed to germinate not one seedling was obtained. Developing ovules were sectioned, but no traces of an embryo were noted, though in some instances there seemed to be an increase in the thickness of either the nucellus or the integuments, or both.

Of all the plants injected, the tobacco showed the greatest response. The ovary is not very large, but considerable liquid can be injected into it without damage, while the larger ovaries of the cucurbits are easily damaged, and ovaries of many plants are too

small. The cucurbit ovaries are very turgid and crack easily, but the tobacco ovaries do not crack or split, which may be due to the presence of more intercellular spaces into which some of the liquid passes and from which it may later diffuse into the cells; the calyx, also, receives some of the injected liquid. It is perfectly possible that the growth substance injected into the calyx and the intercellular spaces of the ovary acts as a reserve which for some time continues to supply the growing cells. In some experiments several injections were made into the same ovary a few days apart. This was done to see if a larger and continued supply of the growth substance would produce a larger fruit, but the ovaries so treated did not grow any faster nor any larger than those receiving only one injection.

A considerable number of tomato ovaries were treated with 5 per cent K-indole acetate paste, and some of these developed into ripe fruits without seeds. Some were of perfectly solid tissue with no trace of locules. However, most of the fruits had well developed locules, but no seeds. The fruits were essentially the same as those obtained with other growth compounds previously reported (Gustafson, 1936).

Cucumbers grown in the greenhouse were both injected and treated with paste. The ovaries injected did not grow into fruits. Sixteen ovaries were treated with the paste, and of these, three developed into mature fruits having an average diameter of 4.6 cm. and a length of 8.33 cm. No seeds or even enlarged ovules were found in these fruits, and the locules were very poorly developed. The thickness of the flesh was greater in these fruits than in those produced by pollination. It is well known that some varieties of cucumbers produce parthenocarpic fruits naturally, but not one of the controls in this variety showed the slightest sign of growth, to say nothing of growing into mature fruits. It might be mentioned in passing that a greater percentage of ovaries treated with 5 per cent indole acetic acid paste grew into fruits than of those treated with its salt, and the fruits were also somewhat larger.

Fig. 1-7.—Fig. 1. Tobacco. On the left are two fruits which were produced by pollination, in the middle is an ovary, and on the right are two fruits which were produced by injecting the ovaries with 1:500 K-indole acetate.—Fig. 2. Tobacco. Cross section of the fruits shown in figure 1. The section on the left is the normal fruit with seeds. The section on the right is the parthenocarpic fruit with partly developed ovules, but no seeds.—Fig. 3. Peppers. The single fruit on the left was produced by treating the cut style with 5 per cent K-indole acetate paste, the next two are normal fruits, and the six on the right were produced by treatment with 5 per cent indole-acetic acid paste.—Fig. 4. Egg plant. Fruits A and B are normal, C is a parthenocarpic fruit produced by treatment with 5 per cent K-indole acetate paste, D and E are parthenocarpic fruits produced by treatment with 5 per cent pyrrole- α -carboxylic acid paste.—Fig. 5. Cross sections of the fruits in figure 4. Notice the seeds in fruits A and B and the lack of seeds in the others.—Fig. 6. Crookneck summer squash. The fruit on the left was produced by cutting off the apical end of an ovary in the flower bud stage, below the locules, leaving no ovules, and smearing the cut surface with 5 per cent indole butyric acid paste. The one in the middle is a normally produced fruit, while the fruit on the right was produced by cutting off the apical end of a young ovary, leaving some ovules, and smearing the cut surface with 5 per cent indole butyric acid paste.—Fig. 7. Crookneck summer squash. At the extreme left is a normal fruit. All the others were produced by cutting off the apical end of the ovary in the flower bud stage and smearing the cut surface with 5 per cent indole-butyric acid paste. The larger ones had more of the ovary left and some ovules.

Peppers grown in the field were also treated with the paste of K-indole acetate. It is well to note here the fact that the percentage of flowers that developed into fruits was usually smaller in the field than in the greenhouse. This was true of hand pollination as well as of other treatments. Of 61 flowers hand pollinated only 4 or 6.5 per cent grew into fruits. One hundred and ten flowers were treated with K-indole acetate paste, and only 2 developed into fruits. This is only 1.9 per cent, and the fruits were somewhat shorter than the normally produced fruits, though the diameter was as great. In figure 3 the single fruit on the left was produced artificially by treatment with K-indole acetate, the next two fruits were produced by pollination, and the six fruits on the right by treating the flowers with 5 per cent indole acetic acid paste. The parthenocarpic fruits had no seeds, and the placentae were greatly reduced. As a cross section figure of a parthenocarpic pepper has already been published by the author (Gustafson, 1937), it is not included here. In one of the fruits produced by the potassium salt and in several of those produced by the indole acetic acid, there were found a number of proliferations issuing from the placentae. These proliferations were about a centimeter in length, several appearing in each fruit. They have not been observed in any fruits produced by pollination. It is an interesting fact that no flowers treated later than July 20 developed into fruits, and only one pollination after this date resulted in fruit setting. The majority of the K-indole acetate treatments were made after this date, which may account for the low percentage of successes. However, natural setting must have occurred later than this date because at the time of the first frost there was an abundance of small fruits on the plants. No control ovaries of the pepper have ever been observed to develop into fruits or even to enlarge.

Only 10 flowers of field-grown egg plants were treated with K-indole acetate, and of these 3 grew into fruits without any seeds. The controls did not grow. Fruit C, in figures 4 and 5 was developed as a result of smearing the cut style with K-indole acetate, while fruits A and B in the same figure are the results of hand pollination. Fruits A and C are of the same age. In fruits A and B the developing seeds are prominent, but no developing seeds are in evidence in fruit C. Further dissection failed to reveal any immature seeds. This dissection was necessary as these were not mature fruits and the seeds of the developing egg plants are not easily observed.

From the above illustrations it is obvious that the potassium salt of indole-acetic acid is active in inducing parthenocarp—perhaps not quite so active as the acid in most instances, but more active on tobacco.

Pyrrrole- α -carboxylic acid.—Of the pyrrrole compounds synthesized the pyrrrole- α -carboxylic acid was

the easiest to prepare, and it was the first one obtained. It was synthesized according to the method of McCay and Schmidt (1926). The melting point of the material used was 205°C. This compound was used on pepper, tomato, egg plant, watermelon, crookneck summer squash, pumpkin, and tobacco. In only three species was there any evidence that this compound acted as a growth substance. Of 24 crookneck summer squashes treated 83.3 per cent showed some growth. The average in length of the ovary was only 59.0 per cent, but of the 15 controls only 33.3 per cent showed any increase, and the average increase of these amounted to only 26.0 per cent. While the increase in growth was very small, it seems that there was, nevertheless, a stimulating action. Ten flowers of eggplant were treated with a 5 per cent pyrrrole- α -carboxylic acid paste, and of these, 5 grew. Some were rather small at the time of picking, but that was due to the fact that they were very young. Fruits D and E in figures 4 and 5 are two of these fruits. Ninety-three tobacco ovaries were injected, and of these ovaries most remained attached to the plant for some time (if unpollinated, tobacco flowers drop off in two days). Eight ovaries remained on the plant for at least a month and more than doubled in size.

Though the results are not very striking, it seems to the author that one is justified in considering that pyrrrole- α -carboxylic acid possesses some growth promoting activity.

Pyrrrole- α -acetic acid.—This compound was the hardest to make and was obtained so late in the season that most of the plants were too old to be used. It was synthesized according to the method described by Nenitzescu and Solomonica (1931). The melting point of the material used was 84°C. Egg plant, pepper, crookneck summer squash, and tobacco were treated with pyrrrole- α -acetic acid. There was no growth in the pepper and only slight growth in the squash, which will be discussed in a later section. Twenty-five eggplant flowers were treated with a 5 per cent lanolin paste, and only 2 grew into fruits. One was 4 cm. long, and the other was 7 cm., when picked three weeks after treatment. This is not very large, but fruits produced by pollination that were a week older measured only 8 to 9 cm. in length. One hundred and forty four tobacco ovaries were injected, and of these 10 per cent made considerable growth, though not so much as ovaries treated with K-indole acetate. The pyrrrole- α -acetic acid can then be said to possess some growth activity.

THE MECHANISM OF FRUIT GROWTH.—*Comparison of rate of growth of fruits produced parthenocarpically by indole-acetic acid and its salt and the rate of growth of normally produced fruits.*—In order to learn something more about the activity of growth substances in fruit development several experiments were devised, but before these are described, some measurements on comparative growth in fruits pro-

TABLE 1. *Comparative rate of growth of fruits of tobacco produced by injecting the ovaries with 1:500 K-indole acetate and by pollination.*

No.	Age in days	Length of fruits in millimeters				Pollinated			
		Treated with K-indole acetate							
		3	5	8	21	3	5	8	33 (ripe)
1	12	14	17			9	16	23	23
2	14	19	23			8	14	20.5	
3	13	18	23.5			8	15	22	
4	12	17	18			10	15	21	22
5	11	16	18			15	17	21	19
6	14	20	24	24					
7	13	15	20			Ave. 10.0	15.4	21.3	21.33
8	12	16	20.5						
9	12	16	20						
10	15	17	20						
11	13	18	21						
12	16	19.5	22.0						
Average		13.0	17.1	20.6					

TABLE 2. *Comparative rate of growth of fruits of tobacco produced by injecting the ovaries with 1:1000 indole-acetic acid and by pollination.*

No.	Age in days	Length of fruits in millimeters		Pollinated	
		Treated with indole-acetic acid			
		5	10	5	10
1	13		14.5	15	23
2	15		17	14	22
3	17		18	17	22
4	13		15	15	20
5	9		9	13	19.5
6	10		12	14	22
7	10		10		
8	10		13	15	22
9	14		15	14	21
10	13		13		
11	14		15	Average 14.6	21.4
12	9		10		
Average		12.25	13.5		

duced parthenocarpically and fruits produced as a result of pollination and fertilization will be noted.

The two tables demonstrate that during the first few days of development the ovaries treated with K-indole acetate grew faster than the pollinated ones, but on the 8th day the latter were growing more rapidly, and the final size was somewhat larger. The average size of 13 ripe fruits produced by pollination gave a measurement of 21.37 mm. in length, while the 12 parthenocarpic fruits in table 1 were only 20.6 mm. in length. Although these latter fruits were

not mature, they were, nevertheless, nearly full grown. The ovaries injected with indole-acetic acid were not measured until they were 5 days old, and at that time they were smaller than the naturally produced fruits, and by the time they were 10 days old this difference was much greater. The fruits produced by indole-acetic acid treatment were always smaller than those produced by pollination and K-indole acetate treatment.

The fact that the ovaries injected with K-indole acetate grew more rapidly at first than the pollinated

ones and later grew slower would indicate that at first they had more available growth promoting material while later it became depleted. On the other hand, in the ovaries of the flowers that had been pollinated there was a continuous renewal of the growth substance, supplied either by the developing seeds or by some other part of the ovary. If the parthenocarpically produced fruits could have had their supply of the growth substances continuously renewed, they might have continued to grow as rapidly as they started out to do. Yet when several injections were made there was no more growth than when only one injection was made.

Influence of developing seeds upon fruit growth.—To discover the influence of seeds upon the development of an ovary into a fruit, two sets of experiments were set up. In the first experiment it was desired to find out if the act of fertilization and the commencement of seed formation was sufficient to enable the ovary to continue its development into a fruit if the young seeds were removed. Crookneck summer squashes that had been insect-pollinated and had made some growth were cut off in such a way that either no ovules or only a few were left in the ovary. As every one familiar with the crookneck summer squash knows, ovules are located only in the apical end of the ovary. In these experiments either all or

many of them might have grown even though they did not contain any ovules nor developing seeds.

The other experiment consisted of reducing the amount of pollination and thus the seed production. Various amounts of the lobed stigma were removed from the unpollinated ovaries of pumpkin and crookneck summer squash before pollination. In some experiments as much as five-sixths of the total stigmatic surface was removed before pollen was applied by hand. In these cases the pollination was thus one-sided. Most of the ovaries treated in this way developed into fruits. A large reduction in stigmatic surface decreased the number of seeds considerably, but even when this happened the fruits were still normal in size and shape. The application of pollen to one side of the ovary did not limit the development of the seeds to that side, and the seed distribution was quite uniform even when as much as five-sixths of the stigma had been removed. It was perhaps to be expected that a reduction in the number of seeds would not decrease the size of the fruits, as it is a common observation that some years apples contain very few seeds, yet they are no smaller than during those years when they produce many seeds. It was expected, however, that the seeds might develop only on that side of the ovary where the pollen was applied and the side without seeds would

TABLE 3. *Influence of growth-promoting substances upon the development of summer squash fruits, when the ovaries were cut so as to eliminate all ovules.*

Treatment	No. of fruits	% that grew	% increase in length	% increase in diameter
Indole-acetic acid	14	71.43	198.02	204.79
Indole-butyric acid	9	77.78	90.55	132.16
Pyrrole-acetic acid	12	8.2	83.33	
Control	6	0	0	

most of the part containing the ovules was cut off. Forty-six young fruits were thus treated. Of these 41.3 per cent continued to grow after the removal of the apical end, but the average increase in length was only 48.23 per cent. On the other hand, some fruits grew considerably more. In fruit number 22 there was an increase of 143.6 per cent, and the length of the fruit at the time of measurement was 38 cm. Another fruit which increased 62.5 per cent had a length of 46.8 cm., which compares favorably with mature fruits. Forty-one normally produced mature fruits were measured, and the average length was found to be 45.0 cm., but they ranged in length from 26.0 cm. to 70.0 cm. It is to be noted that whenever there was marked growth in these decapitated fruits there were a considerable number of ovules present, which were developing into seeds. Though developing seeds seem to be favorable or even necessary to the continued elongation of the fruit, the evidence is not entirely convincing because so many ovaries were destroyed by fungi. It is possible that had these fruits not been attacked by fungi

be apertrophied. There was little, if any, evidence of this. It thus becomes evident that the presence of even a few seeds enables the ovary to make a normal amount of growth, while without any seeds there is little or no growth of the ovary in the crookneck summer squash and none in the pumpkin.

Influence of ovules upon the growth of fruits.—In another series of experiments ovaries from unopened crookneck summer squash flower buds were cut off in a manner similar to that above and the cut surface smeared with 5 per cent indole-butyric acid paste. Of 9 ovaries treated 7 showed some enlargement. The average increase in length was 397.13 per cent, and the fruit on the right in figure 6 grew to a length of 44.0 cm. When these ovaries were treated, no care was taken to exclude all of the ovules, and in several, like the one pictured in figure 6, there were some ovules present, though they did not develop into seeds.

We have no direct evidence that the ovules increase the growth of either the ovary or the fruit, though Katunskij (1936) associates the ovule development

with a high production of auxin in the plant and states that the pre- and post-floral movements of peduncles and scapes of some plants is due to the presence of a large quantity of auxin in the plant, and this auxin he reports is produced in the ovules.

Induction of growth of ovuleless portions of ovaries by growth-promoting compounds.—In further experiments care was taken to exclude all ovules from the remainder of the ovary. In most instances this left about one-third of the original ovary, or a piece about 2 to 3 cm. long and less than one centimeter in diameter. These decapitated ovaries were treated with indole-acetic, indole-butyric, pyrrole-acetic, or pure lanolin. The results of these experiments are given in table 3 and figures 6 and 7.

As the figures and the table show, decapitated ovaries without ovules grew considerably both in length and diameter. The ovule-bearing part having been removed, there was no special enlargement at the apex, and the "fruits" grew with a uniform diameter throughout their whole length. Comparing the percentage increase in growth of ovaries from which all ovules had been removed (when treated with indole-butyric acid) with those in which some ovules were retained, it appears that the presence of the ovules brought about an increased growth beyond that produced as a result of the presence of the indole-butyric acid.

DISCUSSION.—While there is no intention of indulging in a general discussion of growth hormone activity, it may be permissible to draw attention to certain facts that have a broader application than to that of parthenocarp alone. The experiments with the potassium salt of indole-acetic acid corroborate other recent investigations in showing that the acid is not necessary and that at least the potassium salt of indole-acetic acid is very active even though it may turn out to be less active than the acid itself. This has no bearing on the controversy as to the active form of the growth substance in the cell. It is sometimes very convenient to use the salt instead of the acid, as the former is more soluble. Tables 1 and 2 show that the tobacco fruits produced as a result of treatment with the K-indole acetate grew larger than those produced by the acid. This difference in size may be due to the greater concentration of the salt used. The acid was made up to 0.1 per cent, but after standing for some days there was formed a sediment on the bottom of the container so that the concentration used after the first few days was less than 0.1 per cent, and 0.05 per cent solution had previously been found to be too dilute. Therefore the 0.2 per cent solution of the salt gave the plant a greater supply upon which to draw as the

fruit continued to develop. As pointed out before, it is highly probable that the reason the tobacco responded so well was that the solution which went into the sepals acted as a reserve.

While the pyrrole compounds are not so active as the corresponding indole compounds, it is perfectly clear that the phenyl group of the indole radicle is not necessary for the growth promoting activity of indole compounds. The activity of the phenyl group in phenylacetic acid is of course well known, but phenylacetic acid is much less active than indole acetic acid. It is thus seen that the two rings making up the indole radicle possess activity when combined alone with acetic acid, but greater activity when together in form of indole acetic acid. It is to be noted that in the pyrrole compounds used the acid is attached in the alpha position, while in the active indole compounds the acid is attached in the beta position. As far as the writer knows, no indole compounds with the acid in the alpha position have been used as growth promoting substances.

Evidently developing seeds even though few in numbers exert an important influence upon the growth of a fruit. It seems that even ovules without embryos increase the growth of the ovary. Yet ovaries of crookneck summer squash from which all ovules have been removed will grow if growth substances are applied to them, but not without. From all of which one is almost forced to agree with Dollfus (1936) that the ovules growing into the seeds supply the growth hormone, which causes the ovary to become the fruit. But if that is true, where does the hormone come from that is instrumental in the growth of natural parthenocarpic fruit like seedless oranges, grapefruit, grapes, bananas, cucumbers, and others?

SUMMARY

Of 13 new chemicals tested K-indole acetate, pyrrole- α -carboxylic, and pyrrole- α -acetic acids produced parthenocarpic fruits in several different plants. The potassium salt of indole acetic acid is approximately as effective as the acid. The fact that the pyrrole compounds are active proves that the indole radicle is not necessary for growth promoting activity in the indole compounds. Even when the apical part of a crookneck summer squash is cut off, the basal end, without any ovules, will grow if supplied with growth substances. The presence of ovules seems to give a somewhat better growth, however. Ovaries of crookneck summer squash in which seeds have started to grow will not continue to grow if cut in such a way as to eliminate all the developing seeds.

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STRAND FORMATION IN PHYMATOTRICHUM OMNIVORUM¹

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THERE HAS been some discussion in the literature concerning the role of the mycelial strands of *Phymatotrichum omnivorum* (Shear) Duggar in the parasitic action of the fungus. The writers have had opportunity during the last few years to observe the formation of strands under various conditions and believe that an account of the process will be of value in this connection. A brief report of the method of strand formation has been made by Rogers (1932, 1933). The observations presented here relate to the production of strands on the surface of roots of field-infected cotton plants, among the superficial hyphae in pure cultures, and among the network of hyphae produced upon resumption of growth by sclerotia placed in moist chambers.

METHODS.—For the purpose of obtaining information on the time relations in strand formation, a number of Petri dishes containing sterile potato-dextrose agar were inoculated with small masses of hyphae of *P. omnivorum* and incubated at room temperature for one month. During this time the growth of the colonies was observed regularly; at intervals a few of the plates were opened and representative samples of mycelium were removed and mounted in lactophenol for study. A series of drawings was prepared to illustrate early stages in strand formation as shown in these mounts (fig. 5-11).

Small moist chambers were used for observing strand development among the actively growing mycelium which arises from sclerotia and agar blocks containing hyphae. These chambers were prepared by cementing four narrow strips of plate glass one-fourth inch thick in the form of a rectangle upon a thin sheet of clear glass. Canada balsam served as an efficient cement for this purpose. Each of the several cells thus constructed had inside dimensions of approximately two inches by three inches by one-fourth inch and could be sterilized to guard against contamination. Surface-sterilized sclerotia which had been excavated from infested fields were placed in some of these chambers; in others agar blocks con-

taining hyphae were used as inoculum. After insertion of the sclerotium or agar block in each case the chamber was covered by means of a thin glass cover and sealed around the edges with paraffin. The hypha which developed from the inoculum in each chamber grew over the surface of the glass bottom, were observed from day to day, and were photographed at intervals (fig. 1-4).

OBSERVATIONS.—In Petri dish cultures the fungus covers the area of the plate during the first week after inoculation with the typically radiating branch-systems of hyaline, superficial and subsurface mycelium. Each such branch-system is composed of a large central hypha ranging in diameter from 15 μ to 40 μ , with septa at irregular intervals and repeatedly branching lateral hyphae of progressively smaller size. Many of the large hyphae have somewhat thickened walls; in such cases the transverse walls are usually not uniform in thickness, but take the form of a plate of gradually increasing thickness from the center toward the line of junction with the longitudinal wall (fig. 8, 9). In later stages these peripheral thickenings separate centripetally for a short distance and bring about a slight elongation of the hyphae (fig. 10).

The beginning of the compounding of hyphae into strands can be observed in cultures five to ten days old, depending upon environmental conditions, such as type of substratum, moisture, and temperature. Within a branch-system some of the adjacent small hyphae make contact with the central hypha (fig. 2, 3, 5, 6, 10). In either case abundant branching of the small hyphae occurs, with the result that the central hypha is covered with an irregular network of interwoven filaments of much smaller diameter. It should be noted that the agglomeration of small hyphae does not ordinarily proceed uniformly along all parts of the central hypha, but begins with the formation of knots at irregular intervals (fig. 1, 3). From these original knots the compounding of hyphae tends to progress longitudinally until the central hypha is covered at all points. In the formation of a layer the encircling hyphae at first appear as a tangled mass of threads. Later, however, through adjustment of positions and the production of numer-

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ous septa a compact pseudoparenchymatous layer results (fig. 7, 11). The process continues similarly without apparent interruption during the deposition of the second and succeeding layers upon the strand. As Neal, Wester, and Gunn (1934) and others have pointed out, transverse or longitudinal sections of mature strands show an irregular layering of small hyphae around the large central hyphae. The unit strand appears more or less definitely organized,

however, because of the above-mentioned adjustment of positions and the assumption of common septa between adjacent hyphal elements.

As is well known, the mature strands of *P. omnivorum* are buff or brown in color, at least as observed in the field and under ordinary conditions in pure culture, and possess numerous lateral "acicular" hyphae. The buff color of maturity has been noted in strands of various degrees of complexity; occasionally the pigment is visible by the time the second encircling layer is completed. Acicular hyphae are produced by the proliferation of certain cells in the outer two or three layers of the strand (fig. 7). These lateral proliferations may reach considerable length, develop whorled branches, and begin to assume a

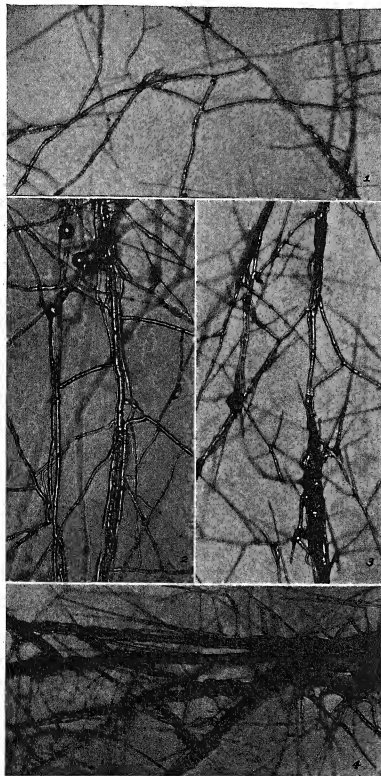


Fig. 1-4. Stages in the development of strands among hyphae produced from sclerotia in moist chambers.—Fig. 1. A portion of the branch-system showing small hyphae beginning to coil around a larger hypha.—Fig. 2. Further association of hyphae.—Fig. 3. Strands forming unevenly along the large hyphae.—Fig. 4. Strands well formed and showing the dark color of maturity. All photographs $\times 90$.

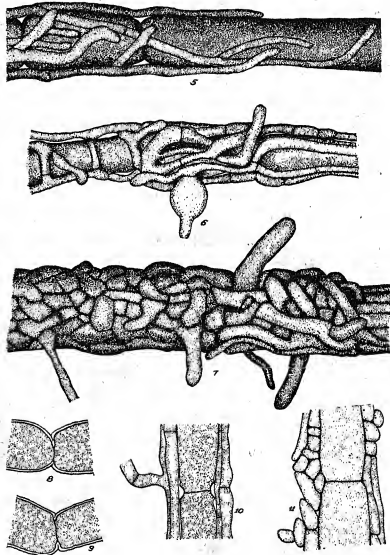


Fig. 5-11. Stages in strand formation in pure cultures on agar plates.—Fig. 5. Small hyphae beginning to grow over the surface of a central hypha.—Fig. 6. Central hypha surrounded by a loose network of small hyphae.—Fig. 7. Deposition of the second hyphal layer.—Fig. 8, 9. Thickenings in longitudinal and transverse walls sometimes observed in large hyphae.—Fig. 10. A portion of an optical longitudinal section of a developing strand showing separation of the thickening in the septum of the central hypha.—Fig. 11. A portion of an optical longitudinal section of a young strand showing pseudoparenchymatous outer layers. All figures $\times 462$. The hyphae represented in Fig. 5-11 were hyaline, except in Fig. 7 and 11, in which the exterior cells at least were pale brown.

transparent yellow or light brown color. At this time the acicular hyphae become thin and present the aspect of more or less rigid spines. During the third week after inoculation the colonies on agar plates appear uniformly buff-colored, and microscopic examination shows a large number of mature strands of various degrees of complexity; at this time there is little evidence of the initiation of new strands.

DISCUSSION.—Previous work has shown that cotton seedling roots inoculated with *P. omnivorum* under pure culture conditions are invaded within five days by the rapidly growing hyaline mycelium (Watkins, 1938a). In such cases strands are not usually formed until the eighth or tenth day. Similarly in natural infections of cotton plants in the field the lesions on the roots are caused by the activity of vigorously growing hyphal tips, which in the majority of cases are not immediately associated with strands (Watkins, 1938b). Strands in developmental stages are sometimes seen on the exterior of advanced lesions. These observations suggest that compound hyphal structures are produced by the fungus when it approaches maturity and are perhaps formed only after the mycelium has become thoroughly established in a given site. The strand is apparently not necessarily a direct precursor of hyphal invasion.

SUMMARY

A study of strand formation on the roots of cotton plants infected with *Phymatotrichum omnivorum*

(Shear) Duggar, in pure cultures of the fungus, and among hyphae developing from sclerotia show the process to be similar under all these conditions. The mycelium is composed of large hyphae with numerous repeatedly ramifying lateral hyphae of progressively smaller diameter. Some of the smaller hyphae make contact with the larger at irregular intervals and begin to grow over their surfaces. The process continues until the central hypha in each case is covered with a more or less compact pseudoparenchyma. Subsequent layers of cells are deposited in the same way upon the strand.

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A COMPARATIVE HISTOGENETIC STUDY OF THE BUD SCALES AND FOLIAGE LEAVES OF VIBURNUM OPULUS¹

G. L. Cross

DURING RECENT years bud scales have been studied rather intensively with respect to the nature of their homology with foliage leaves. The idealistic "Doctrine of Metamorphosis" developed by Goethe (1790), the suggestion of an ontogenetic metamorphosis advanced by Goebel (1880, 1932), and other theories purporting to interpret the bud scale have been critically analyzed by Foster (1928), who has stressed the fact that additional information must be obtained before definite conclusions can be drawn.

Goebel's conception of the bud scale—i.e., that it represents an ontogenetic metamorphosis of the primordium of a foliage leaf—has been shown to be inapplicable to certain highly specialized cataphylls such as those of *Carya Buckleyi* var. *arkansana* (Foster, 1935) and *Morus alba* (Cross, 1936, 1937a). The latter researches and those of Schiepp (1929) indicate that many highly specialized cataphylls commonly show a pronounced acceleration of marginal growth correlated with reduced growth in radial thickness; while in contrast a salient feature of the

early development of foliage leaves is that of an accelerated growth in radial thickness through the activity of an adaxial meristem. These differences may be detected in *Carya Buckleyi* var. *arkansana* and in *Morus alba* when the primordia are very young (80-100 μ high).

In an effort to determine whether this behavior is also characteristic of forms with less specialized cataphylls, an investigation of *Viburnum rufidulum* Raf. was recently undertaken (Cross, 1937b). It was found in this species that the cataphylls develop much like foliage leaves during their earlier stages, and, except for a copious production of epidermal hairs, are not distinguishable from foliage leaves of a comparable size up to the 500 μ stage. After this stage somewhat divergent developmental features were noted. This limited evidence indicates that the developmental features, found in the highly specialized cataphylls of *Morus alba* and *Carya Buckleyi* var. *arkansana* are not characteristic of the less specialized bud scales of *V. rufidulum*, and the question arises as to the nature of the growth processes in the cataphylls of other forms. In order to evaluate the significance of the results obtained from *V. rufidulum*

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it seemed desirable to make a comparative study of some related species with highly specialized cataphylls. The present study of *V. Opulus* (highly specialized cataphylls) has been undertaken in an effort to determine what histogenetic processes are associated with the differences in structure of its cataphylls and foliage leaves and to compare the data with those previously obtained from a similar study of the less specialized bud scales of *V. rufidulum*. As a matter of somewhat secondary importance, the origin and development of the peculiar glands and stipular appendages found on the petiole of the foliage leaf have been briefly studied.

METHODS.—Materials for the investigation were obtained from various specimens of *V. Opulus* grown ornamentally in Norman, Oklahoma, during the season of 1936. Collections were made every third day during the period of rapid bud expansion, and weekly thereafter. The most successful fixing reagent proved to be a solution of 5 per cent commercial formalin and 6 per cent glacial acetic acid made up in 70 per cent ethyl alcohol. For dehydration and clearing, a series of tertiary butyl alcohols was used. The materials were imbedded in paraffin (56°C.), and sections 10 μ . in thickness were cut. Safranin and Fast Green F.C.F. were used in staining, and the Fast Green was applied from a solution in xylol and absolute alcohol (Cross, 1937c).

THE SHOOT.—The winter twigs of *V. Opulus* are slender, with lateral buds and opposite, crescent-shaped leaf scars. Shortly after the shoot is fully expanded, the terminal portion dies back; therefore terminal buds are not to be found on mature shoots. Leaf buds and mixed buds occur on all twigs, but the latter are easily distinguished by their greater size. The outer components of each bud are two highly specialized, scale-like, opposite, glabrous, connate cataphylls (fig. 1). Immediately within and alternating with the cataphylls is a pair of opposite, somewhat pubescent, connate transitional forms. In each leaf bud usually four pairs of foliage leaves are found—the youngest pair in a primordial stage of development. The vernation is plicate as a result of the conuplicate folding of the lobes of the leaves.

When bud expansion occurs the inner components are the first to begin growth. The cataphylls separate, and the tips of the transitional forms become visible. The transitional forms vary considerably in structure. Frequently they seem to consist of vaginate "petioles" surmounted by minute three-lobed blades (fig. 2), or there may be no trace of a blade. The foliage leaves soon appear, and when completely expanded, are petiolate with nearly glabrous, deeply three-lobed, acuminate blades (fig. 5). While only four pairs of foliage leaves are found within the bud, additional ones are formed by the apical meristem during bud expansion, and as many as seven pairs may appear on the mature twig. "Stipular" appendages are formed near the base of the petiole (fig. 3, 5), and occasionally additional ones appear along the margins of the petioles. Glands are produced on the

petiole near the blade and sometimes on the base of the blade (fig. 4). The cataphylls do not appreciably increase in size as the bud expands—in contrast to the behavior of the cataphylls of *V. rufidulum*; but the transitional forms may double or triple their original length. The internode between the cataphylls and transitional forms does not elongate. The internode between the transitional forms and the first pair of foliage leaves elongates, but usually somewhat less than the succeeding internodes. In 1936 the buds of *V. Opulus* had begun to expand by March 14. In 1935 the buds of *V. rufidulum* (Cross, 1937b) had begun expansion during the last week of February; in 1937, by March 1.

One of the earliest descriptions of the bud of *V. Opulus* is that of Henry (1846), who noted the presence of stipular appendages (Nebenblättchen) on the older, enclosed foliage leaves. Henry listed this species in the section of his paper entitled "Nebenblattdeckige Knospen, Gemmae stipulaceae," although he did not discuss the importance of the stipules in the construction of the scales. Lubbock (1899) stated definitely that: "Each bud is covered by two pairs of scales, which are modified petioles bearing just a trace of an undeveloped lamina at the apex." His

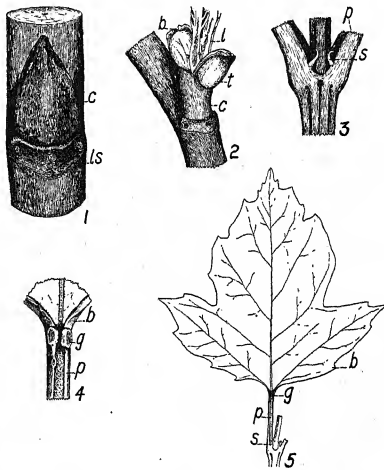


Fig. 1-5.—Fig. 1. Dormant axillary bud of *Viburnum Opulus*; c, cataphyll; ls, leaf scar. $\times 4$.—Fig. 2. Expanding shoot; c, coalesced cataphylls; t, transitional form; b, terminal lobes of transitional form; l, foliage leaf. $\times 2$.—Fig. 3. Node of young twig; p, petiole; s, stipular appendage. $\times 2$.—Fig. 4. Juncture of petiole and blade of young leaf; g, gland; b, blade. $\times 2$.—Fig. 5. Leaf and portion of twig. $\times 1$. (Figures 1-5 drawn by Virgil Johnson).

interpretation of the homology of the bud scale was derived from external studies of the adult cataphylls and is perhaps open to question. Foster (1928) mentioned the "danger of constructing hard and fast morphological definitions of bud scales." He thought it unnecessary to attempt to homologize every type of bud scale with some portion or portions of an ancestral foliage leaf, and he suggested that it might be more correct to regard many scales which show external conformation to parts of foliage leaves as examples of homoplasy rather than of direct homology. A somewhat similar view was expressed recently by Arber (1937), who objected to the term "homology" except in an abstract sense, and who suggested that the term "parallelism," referring to "parallel development," might be substituted. Further reference to these views will be made later.

The stipular appendages and glands of *V. Opulus* have stimulated considerable discussion. In 1861 Bailon interpreted the stipular appendages as representing lobes of the leaf. Lubbock (1899) noted: "The peculiar filiform stipuliform appendages, nothing exactly resembling which occurs in any of our other forest trees, the nearest approach being in the allied genus *Sambucus*. The presence of stipules in *Viburnum* would be the more remarkable, as in the family *Caprifoliaceae*, to which the *Viburnums* belong, stipules (if they be stipules) are confined to this genus, to *Pentaptyxis*, and to *Sambucus*." He likewise remarked: "The stipuliform appendages resemble leaf-lobes in being slightly conduplicate," and mentioned "two or more honey glands at the base of the lamina" of the foliage leaf. From this one gathers that Lubbock rather doubted the stipular nature of the "stipular appendages."

Goebel (1932) regarded the stipular appendages as stalked glands which, because of their position, are termed "stipules." He based his interpretations upon the fact that the stipular appendages frequently bear terminal glands; and he fancied that he was able to see transitional stages between the sessile glands of the upper petiole and the "stipules." However, Lubbock (1899) said of the "stipules": "These often bear honey-glands, but by no means always, and even where these occur there seems no reason why they should be situated on filiform appendages. . . . I doubt whether they can be explained as mere honey glands. . . ." Evidence gleaned from the present investigation is in support of the views of Lubbock rather than Goebel.

THE ANATOMY OF THE FOLIAR BUD.—Only leaf buds were used for anatomical studies, because the inflorescences in the mixed buds so distort the leaves and axis as to make impossible accurate orientation for cutting. Figures 6 to 12, prepared from transsections of a dormant bud of *V. Opulus* about 3 mm. long, will be useful in understanding the grosser anatomical features of the bud in this species. Figure 6 shows the cataphylls about 50 μ below their apex. Three

vascular strands are in each cataphyll, a fact previously reported by Lubbock (1899), who regarded

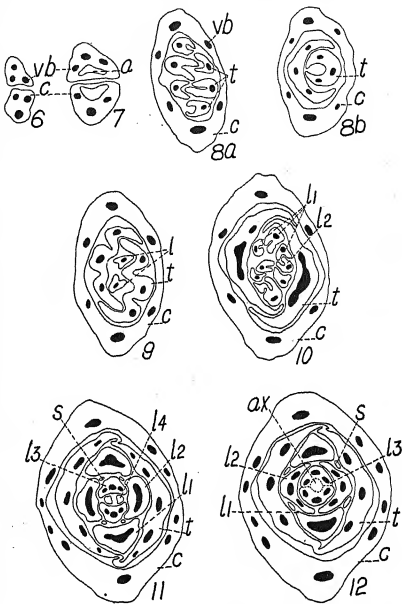


Fig. 6-12.—Fig. 6. Transsection of dormant axillary bud about 50 μ below apex; c, cataphyll; vb, vascular bundle. $\times 15$.—Fig. 7. Transsection of bud about 200 μ below apex; a, air space. $\times 15$.—Fig. 8a. Transsection of bud about 775 μ below apex; t, lobes of a transitional form. $\times 15$.—Fig. 8b. Transsection of bud about 780 μ below apex; t, transitional form without terminal lobes. $\times 15$.—Fig. 9. Transsection of bud about 1050 μ below the apex; l, tips of first pair of foliage leaves. $\times 15$.—Fig. 10. Transsection of bud about 1440 μ below the apex; l, lobes of one of the first formed pair of foliage leaves; l, tip of one of the second pair of foliage leaves. $\times 15$.—Fig. 11. Transsection of bud about 2335 μ below apex; l, petiole of first pair of foliage leaves; l, petiole of second pair of foliage leaves; l, petiole of third pair of foliage leaves; l, primordium of fourth pair of foliage leaves; s, stipular appendage of second pair of foliage leaves. $\times 15$.—Fig. 12. Transsection of bud about 2540 μ below apex; ax, nonvascular portion of axis surrounded by basal portion of the third pair of foliage leaves; s, stipular appendage of first pair of foliage leaves. $\times 15$.

trary, the tip is commonly recurved adaxially in such a manner as to form a hollow, hood-like apex (fig. 7). The recurving occurs early in the ontogeny, and the process will be described later. The cataphylls are entirely glabrous; but their abaxial surfaces are covered by a heavy layer of cutin, which at maturity may be nearly as thick as the radial dimension of an epidermal cell (fig. 25). They are coherent at their margins to within 350–400 μ of their apices.

The transitional forms here shown (fig. 8a) exhibit relatively well developed trilobate blades which have attained a length of about 1000 μ . The petioles were about 1800 μ in length. However, the extent of development of the blade varies greatly, and, as noted before, the blade may not appear at all (fig. 8b). The transitional forms are not cutinized, but they develop abundant abaxial and less numerous adaxial epidermal hairs. They cohere by their margins for from one-third to two-thirds of their length in the dormant buds (fig. 11, 12). The cataphylls and transitional forms of *V. Opulus* show neither external nor anatomical evidence of the presence of stipules.

The first pair of foliage leaves in the resting bud of *V. Opulus* is well differentiated, with blades approximately 1400 μ and petioles about 170 μ in length (in buds about 3 mm. long). Embryonic stipular appendages, without differentiated vascular strands are present on each margin near the base of the petiole (fig. 12), but the glands, so characteristic of the adult leaf, have not formed. The second pair of foliage leaves is likewise clearly differentiated into a blade and petiole, with very small stipular appendages near the base of the latter (fig. 11). The third pair of foliage leaves, while clearly differentiated into a trilobate blade and a petiole, has not yet produced its stipular appendages. From this it is clear that the stipular appendages are produced subsequent to the differentiation of the blade and petiole, a situation not in keeping with the usual behavior of stipules, for stipules frequently arise early in the ontogeny of the leaf, often before the leaf blade (Miksch, 1876; Lubbock, 1899; Cross, 1937a). Eichler (1861) has stated: "Die Entstehung der Stipulä aus dem Blattgrund ist das wesentlichste Moment zu ihrer Charakterisierung." It seems evident that the stipular appendages here may represent the lowest lobes of a basipetally developing leaf. That they are true stipules in the sense that Eichler used the term seems doubtful despite Baillon's (1861) contention that *Viburnum* provides "une bonne demonstration de le fait que les stipules son des lobes de feuille."² It should be

² It seems unfortunate that the meaning of the term "stipule" has never been clarified. Schiller (1903) attempted to distinguish between true stipules and pseudostipules on a basis of their origin. He regarded appendages which diverge from the leaf base as true stipules (eigentlichen Stipulae), while those which are formed higher on the leaf-axis he termed pseudostipules (Pseudostipulae). However, as it is used in the modern sense (Goebel, 1932), the term "stipule" apparently may be applied to any appendage upon or near the base of the leaf, and its use is without morphological significance.

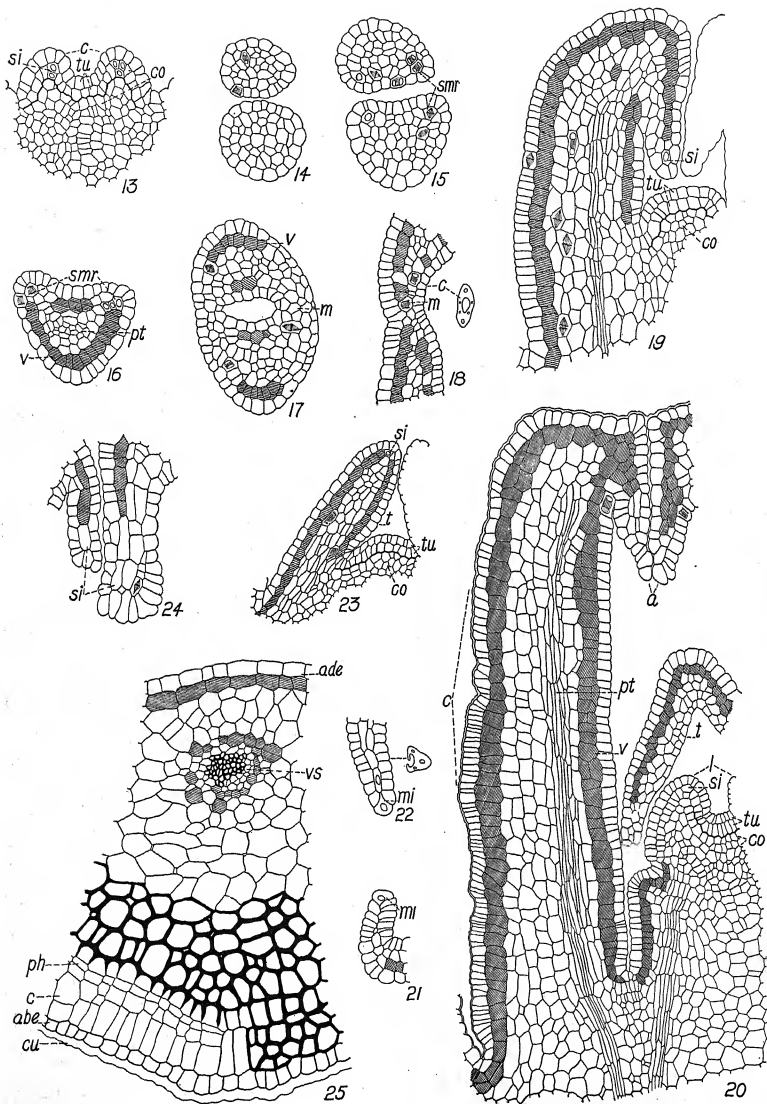
noted also that the stipular appendages are initiated prior to the formation of the glands.

The fourth pair of foliage leaves in the resting bud is represented only by a pair of undifferentiated primordia (fig. 11) which are ordinarily about 30 μ in height.

The structure of the axis of the bud of *V. Opulus* is similar to that of *V. rufidulum* (Cross, 1937b). It consists of peripheral confluent leaf bases arranged around a central non-vascular core (fig. 12). In the case of the cataphylls and transitional forms, the confluence is continued above the plane at which these organs diverge from the axis (fig. 8–12). No cauline vascular or provascular tissue could be demonstrated at any level in the axis of the bud, all strands being formed basipetally in continuity with previously differentiated leaf traces. There is, then, no fundamental morphological distinction between the "stem" and "leaf" of *V. Opulus*, although these terms are definitely of practical value. The "growth unit" theories of Louis (1935), Gregoire (1935a, 1935b) and Priestley (Priestley, Scott, and Gillett, 1935) are useful in understanding the structure of the bud (Cross, 1937b).

THE ORIGIN OF THE AXILLARY FOLIAR BUD AND THE GROWTH OF THE CATAPHYLL.—The axillary buds of *V. Opulus* arise during the period of bud expansion. The bud is initiated by periclinal divisions in the outer several layers of the axis, although the extent of the axis that is involved, in terms of actual numbers of cell layers, was not determined. The apical meristem of the new axis is formed first and, due perhaps to its crowded quarters, assumes the form of an elongated rectangle with the greater dimension in the plane which approximately bisects the angle formed by the subtending leaf and the axis of the parent twig. The lateral portions of the rectangle form the "foliar buttresses" (Louis, 1935; Gregoire, 1935a, 1935b; Cross, 1937b) from which the primordia of the cataphylls arise in a nearly vertical position (fig. 13). Each "foliar buttress" with the corresponding cataphyll may be regarded as a growth unit (fig. 13), which, with the growth units to be formed later as the transitional forms and foliage leaves develop, will constitute all of the bud except the central non-vascular portion of the axis. The dimensions of the apical meristem just prior to the formation of the cataphylls are approximately 25 μ by 100 μ , although considerable variation was noted.

Studies of numerous buds indicate that at this stage the apical meristem consists of a two-layered tunica and a central corpus (fig. 13). This seems of special interest since a four-layered tunica was found in *V. rufidulum*. The contributions of the tunica and corpus to the primordia of the cataphylls is clearly shown in figure 13, where the corpus extends like a core into the base of the primordium on the right. Near the apex of this primordium one of the cells of the inner tunica layer has divided by the insertion of a periclinal wall. The outer derivative has divided in turn by an anticlinal wall. Studies involving



longisections of later stages indicate that the apical growth of each cataphyll primordium is dominated, at least for a time, by a single subapical cell or small group of cells which divide alternately in anticlinal and periclinal planes in the same manner as do the corresponding subapical initials in the cataphylls, transitional forms, and foliage leaves of *V. rufulum*.

The first indications of cell specialization appear very early when the primordium is less than 75 μ high. Certain abaxial subepidermal cells (derivatives of T-2) near the median basal portion of the primordium become highly vacuolate and stain heavily with Safranin. As the primordium increases in size, the area of cells behaving in this manner is extended vertically and laterally. Soon the adaxial subepidermal layer is similarly vacuolated, and finally only the apical and marginal cells of the subepidermal layers remain unvacuolated (fig. 16, 17, 19).

When the cataphylls have attained a height of approximately 100 μ , before the primordia of the transitional forms have appeared, the portion of the apex of the axis between each pair of opposing margins produces a connecting ridge of tissue, and then by zonal growth³ a tubular base common to both cataphylls is formed (fig. 17). At no stage in ontogeny could evidence of the differentiation of a blade and petiole be demonstrated.

Rapid division of the cells of the abaxial epidermal layer and the enlargement of the cells of the rest of the abaxial portion thrust the tips of the cataphylls together at an early stage, after which apical growth of the primordia may be directed downward towards the apex of the bud-axis (fig. 19, 20). These downwardly directed apices may become somewhat large and glandular, and the subapical cells continue to divide until the scales are about 1 mm. long (fig. 24).

Scales 200–500 μ high frequently show interesting variations in apical growth. The variations are associated largely with the behavior of the epidermal layer. In young scales the epidermal layer divides

³ The zonal growth in the development of the paired cataphylls of *V. Opulus* is suggestive of that described for the bifoliar prophyll of *Carya Buckleyi* var. *arkansana* (Foster, 1932); however, the prophyll of *Carya* is initiated by the elevation of a peripheral, prophyllary ring, upon which the apices of the lateral portions are differentiated.

only anticleinally, and distinctive apical initials could not be demonstrated. In older scales, however, the apical epidermal cells often deviate from the ordinary mode of division—frequently dividing by alternating oblique walls so that a biseriate layer of cells is formed. These layers diverge from the apical row (fig. 20) in a manner suggestive of the cataphylls of *Morus alba* (Cross, 1936). Finally the apical row may divide strictly parallel to the apex thus producing a uniseriate layer (fig. 20), a phenomenon also described for *Morus alba*. The plasticity of the apical and marginal initials of foliar organs with respect to variations in the planes of division was first emphasized by Lund (1872). Recently Foster (1937) has described the complexity in the behavior of the marginal initials in the bud scales of several forms of *Rhododendron*. The evidence thus clearly indicates that departures from the usual anticlinal plane of division of the marginal or apical initials may be widespread in the foliar organs of Angiosperms.

Tissue differentiation proceeds with great rapidity, provascular strands being well formed in scales 100 μ high, and air spaces in those 300–500 μ high (fig. 19). One outstanding characteristic is the extraordinary enlargement of the cells of the abaxial portion. The dimensions of these cells increase two or three times, as shown in figures 19 and 20, and although they round off and form air spaces, they continue to divide rapidly. Figure 20 shows evidence of excessive anticlinal division in the abaxial epidermal layer, with relatively less mitotic activity in the adaxial layer.

Marginal growth of the cataphyll.—After approximately the 100 μ stage, when the bases of the opposite cataphylls have become continuous as a result of zonal growth, two regions of marginal growth are to be considered—i.e., the free margins of the upper cataphylls and the common undiverged margins of the lower tubular portion. The free margins of each upper cataphyll grow for a time as described for *V. rufulum* (Cross, 1937b), by the alternating periclinal and anticlinal divisions of a row of submarginal initials, by divisions of the derivatives of the submarginal row, and by anticlinal divisions of the marginal cells (fig. 14, 15, 16). Later when the cataphylls have attained a size of 1000–1500 μ , the free margins may be extended by divisions perpendicular to the

Fig. 13–25.—Fig. 13. Origin of young axillary bud showing primordia of cataphylls; *c*, cataphylls; *st*, subapical initial of cataphyll; *tu*, tunica layers; *co*, corpus. $\times 165$.—Fig. 14. Transsections of pair of cataphylls (115 μ high; about 75 μ from base). $\times 165$.—Fig. 15. Transsection of pair of cataphylls (115 μ high; about 45 μ above base); *smr*, submarginal rows of initials. $\times 165$.—Fig. 16. Transsection of cataphyll (about 160 μ high; about 50 μ above base); *pt*, provascular tissue; *v*, vacuolated cell. $\times 165$.—Fig. 17. Transsection of pair of confluent cataphylls (150 μ high; about 15 μ above the base); *m*, confluent margin. $\times 165$.—Fig. 18. Transsection of pair of confluent margins of a pair of cataphylls (600 μ high) about 400 μ below the tip; *m*, marginal initial. $\times 165$.—Fig. 19. Longitudinal section of cataphyll with tip reflexed toward apex of stem; *st*, subapical initial of cataphyll. $\times 165$.—Fig. 20. Longitudinal section of older cataphyll, portion of transitional form, and young leaf; *v*, apex of cataphyll; *pt*, provascular tissue; *t*, transitional form; *l*, first pair of leaves; *st*, subapical initial of foliage leaf. $\times 165$.—Fig. 21. Transsection of margin of cataphyll (about 1600 μ high; 600 μ from apex); *mt*, marginal initial. $\times 165$.—Fig. 22. Transsection of margin of cataphyll (about 1600 μ high; 650 μ from apex). $\times 165$.—Fig. 23. Longitudinal section of young transitional form and portion of apical meristem; *st*, subapical initial; *t*, transitional form. $\times 165$.—Fig. 24. Longitudinal section through apical portions of a pair of cataphylls about 1 mm. high. $\times 165$.—Fig. 25. Transsection of the median basal portion of a mature cataphyll; *ade*, adaxial epidermis; *vs*, vascular strand; *ph*, phellogen; *c*, cork cells; *abe*, abaxial epidermis; *cu*, cuticle. $\times 165$.

surface in the submarginal initials, accompanied by antinodal divisions in the surface cells, producing a layer three cells thick. In such cases, distinctive marginal initials may also be recognized (fig. 22). These may divide exclusively by antinodal walls; or the walls may be inserted at oblique angles in such a manner as to produce a biserial marginal layer (fig. 21) resembling that described for the bud scales of *Morus alba* (Cross, 1936) and *Rhododendron* (Foster, 1937). Periclinal divisions were not found in the marginal initials of the cataphylls, although it is not unlikely that these may occur.

The tubular base, common to both cataphylls, possesses lateral vertical regions which may be regarded as undiverged margins. Evidence supporting this interpretation is supplied by the fact that the circumference of the tubular portion is increased by the active periclinal and antinodal divisions of vertical rows of cells that extend the entire length of the tube on either flank (fig. 17, 18) and which apparently are the equivalent of marginal and submarginal initials. The cells of these regions remain small, undifferentiated, and actively mitotic for some time after the cells elsewhere in the tube have enlarged and become highly vacuolated.

Anatomy of the mature cataphyll.—The structure of the mature cataphyll is of interest because of its extremely specialized tissues. A few brief statements concerning the anatomy of the bud scales of *V. Opulus* have been made by Schumann (1889), although no figures were included in his account. A somewhat more detailed description of the bud scales (no figures) of *V. dentatum* has been provided by Brick (1914). The transection represented in figure 25, although through only the region of the midvein, is representative of the tube formed by the undiverged cataphylls about 1 mm. above their bases.

The abaxial epidermis is covered by a layer of cutin which is nearly as thick as the radial diameter of an epidermal cell. This cuticle is continuous over the entire bud, becoming confluent at the junctures with the cutin of the oppositely placed cataphyll (fig. 20), and forming a layer as nearly impervious to moisture as it would seem possible to construct. The walls of the epidermal cells are somewhat thickened by deposits of cellulose, and occasional ones show scalariform pitting. All are filled with densely staining granular material.

The tissue immediately within the abaxial epidermal layer consists of greatly enlarged cells with enormously thickened walls. The thickening material is apparently cellulose, and scalariform pits are abundant, though not shown in figure 25. The thick-walled cells usually extend inwardly for about one-half of the radial dimension of the cataphyll. Chloroplasts are largely restricted to this tissue.

Occasionally, before the thickening material is deposited, the subepidermal layer may undergo localized, periclinal divisions so that an incipient cork cambium and two layers of cork cells are produced (fig. 25). A very similar behavior has been reported

by Brick (1914) for *V. dentatum*. Apparently in neither of these species does the cork cambium become active and produce extensive layers of cork cells such as have been described for *Aesculus neglecta* and other species (Mikoseh, 1876).

Between the region of the thick-walled cells and the adaxial epidermis is a tissue consisting of large, thin-walled, irregularly-shaped cells which are practically devoid of chloroplasts. Each cell contains a large crystal (not shown in the figure). The vascular bundles occur in this tissue. They consist of numerous xylem cells surrounded by small, thin-walled cells which probably function as phloem. Sieve tubes and companion cells could not be demonstrated, which is in agreement with Brick's (1914) account of *V. dentatum*. An interrupted sheath of densely staining, rather small thin-walled cells surround the vascular bundle.

The adaxial subepidermal layer consists of the vacuolated, densely staining cells apparently characteristic of the genus. The adaxial epidermis has thickened, pitted walls; but the contents of the cells are not densely staining as are those of the abaxial epidermis. Cutin is not secreted by the adaxial epidermis. Stomata were not found in either epidermal layer, although they have been reported for the genus by Schumann (1889). The cataphyll of *V. Opulus* is clearly a highly specialized structure with a function almost exclusively of protection.

THE TRANSITIONAL FORM.—The variable nature of the transitional form has already been mentioned. The following account is by no means comprehensive, but is thought that a few rather general remarks might be of interest. The primordia of the transitional forms appeared in the lateral buds during the first week in April when the cataphylls were approximately 200 μ high, indicating that the first plastochron is about two weeks in length. Both tunica and corpus contribute to the primordia, and no outstanding departure from the mode of initiation of the cataphylls was observed. Apical growth of the type described for the young cataphylls obtains at an early stage and apparently persists until the transitional forms are at least 75 μ high (fig. 23). The later stages of development vary remarkably, doubtless in correlation with the variation in final form of the organ. The histogenesis of the transitional form is so variable and complex as to deserve treatment in a special paper.

Ordinarily the transitional forms grow rapidly in a vertical direction but do not increase much in radial thickness. They therefore appear somewhat more slender than cataphylls of a comparable height (fig. 23).

At a height which varies greatly zonal growth is initiated, and a common, tubular basal portion is produced. The variation in height at which zonal growth begins is apparently related to the presence or absence, or to the extent of development, of a blade. Transitional forms which have a very small blade or none at all at maturity, develop zonal growth at a very early stage in their ontogeny and are otherwise

histogenetically similar to the cataphylls. On the other hand, transitional forms which will develop a conspicuous blade exhibit a somewhat delayed zonal growth and show other histogenetic characteristics suggestive of the cataphylls, transitional forms, and foliage leaves of *V. rufidulum* (Cross, 1937b). In this case the most suggestive histogenetic divergence from the cataphyll is the development of an adaxial meristem in the petiolar region.

Marginal growth of the upper free portions of the transitional forms is at first quite like that described for the cataphyll. Later, in the distal regions of the ones which do not develop blades, or only rudimentary blades, interesting variations in marginal growth may be seen. The marginal epidermal cells, which ordinarily divide only in anticlinal planes, frequently divide by alternating oblique walls, thus forming a biseriate layer of cells similar to that formed at the margin of the cataphyll and at the apex and margins of the cataphyll of *Morus alba* (Cross, 1936). Marginal cells of this type have been discussed by Foster (1937) in a recent paper on the bud scales of *Rhododendron*. Later, in many instances the marginal initials may divide by walls parallel to the margins, thus producing a uniseriate plate of cells along either edge of the transitional form. Transitional forms which develop lobed blades have submarginal rows of initials that function like those in the foliage leaf of *V. rufidulum* (fig. 43, 44); however, they cease division and mature before an extensive lamina is produced. No evidence was obtained that these initials are active during the period of bud expansion.

THE FOLIAGE LEAF.—Initiation of the foliage leaf.—The primordia of the first pair of foliage leaves appeared in 1936 when the transitional forms were about 200 μ high and the cataphylls about 500 μ high. They formed in the various lateral buds during the third week in April, indicating that the second plastochron is about equal in length to the first.

Stem apices from which foliage leaf primordia are about to diverge show a distinctive staining reaction in that the Safranin is difficult to differentiate and shows a tendency to remain in the cytoplasm. This tendency to stain densely with Safranin could not be demonstrated in apices just prior to the formation of cataphylls and transitional forms; and it may be indicative of a chemical change in the apical meristem which is associated with foliage leaf formation. Similar densely staining tissue has been reported for the stem apices and young foliage leaves of *Carya Buckleyi* var. *arkansana* by Foster (1935) who says: "A subtle yet important indication of the early divergence of the foliage leaf primordium consists in the deeply stained character and extremely rapid rate of division of its cells." This staining reaction is so marked in *V. Opulus* that one has no difficulty in distinguishing between the primordia of the youngest cataphylls and foliage leaves, or between the apices before the primordia appear.

The preliminary phases in the initiation of a pair of foliage leaves involve periclinal divisions in the second

layer of the tunica and the outer layers of the corpus. The outer layer of the tunica divides only in anticlinal planes, thus maintaining its individuality during the entire ontogeny of the leaf. The inner tunica divides periclinal forming two layers of derivatives. From the outer of these layers, at the subapex of the primordium, an area of localized apical growth is organized at a very early stage (fig. 20). The identity of the inner layer derived from the inner tunica is soon lost. A study of the tips of numerous primordia indicates that apical growth is dominated by a single initial which divides irregularly in periclinal and anticlinal planes (fig. 20, 26, 31). However, much of the early increase in height of the primordium is due to the intercalary growth. The extent of the contribution of the corpus to the primordium could not be determined with certainty, although from figures 20 and 26 it seems evident that derivatives of the corpus extend as a core into the base of the petiolar-midrib region.

The young leaf grows rapidly in a vertical direction. Excessive growth of the abaxial surface thrusts the tip slightly over the apex of the stem-axis and the tips of oppositely placed leaves may finally establish contact.

Differentiation of the midrib, petiole, and base.—Except for the differences in staining, the primordia of the foliage leaves somewhat resemble those of the cataphylls and transitional forms until a height of approximately 80 μ has been attained (measuring along the adaxial surface). At this stage certain median, proximal, adaxial subepidermal cells undergo periclinal divisions (fig. 26). The derivatives of these cells continue to divide, forming an adaxial meristem of the type described for many species of Angiosperms (Bouygues, 1902; Foster, 1935; Cross, 1937b). Since a comparable meristem was not found in the primordia of the cataphylls at any stage, this phenomenon serves as a subtle, but critical, criterion of distinction. A similar histogenetic divergence between cataphyll and foliage leaf primordia has been reported by Foster (1935) to occur at the 90 μ stage in *Carya Buckleyi* var. *arkansana*.

In addition to serving as a critical method of distinguishing the primordia of foliage leaves and cataphylls, the formation of an adaxial meristem is of further significance in that it provides the first indication of the differentiation of a base, petiole, and midrib. The enlargement of the derivatives of the adaxial meristem causes a slight swelling on the adaxial surface which supplies the first external evidence of a petiole. In figure 31 is shown a longitudinal section of a young leaf which has differentiated into a terminal midrib, a median, adaxially swollen petiole, and a lower basal portion. Comparable modes of petiole-midrib differentiation have been described by Schüpp (1929) for *Acer pseudoplatanus*, by Foster (1935) for *Carya Buckleyi* var. *arkansana*, and by Cross (1937b) for *Viburnum rufidulum*. The phenomenon is probably of widespread occurrence in Dicotyledons.

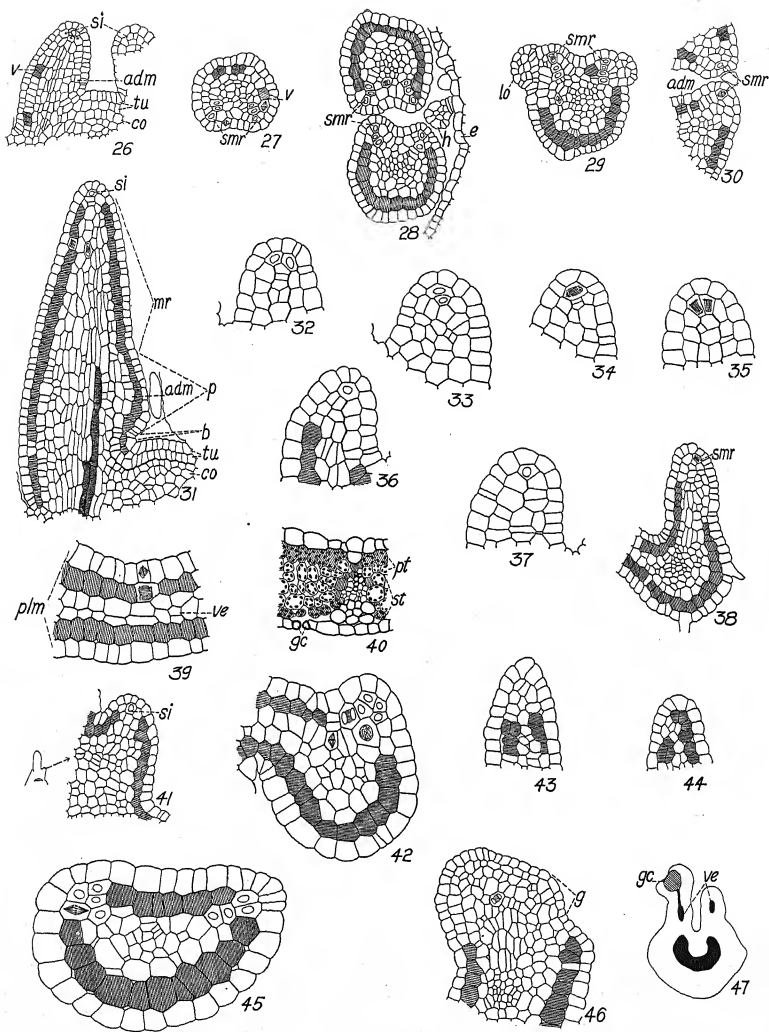


Fig. 26-47.—Fig. 26. Longitudinal section of young leaves and apical meristem; *si*, subapical initials; *v*, vacuolated cell; *adm*, adaxial meristem; *tu*, tunica; *co*, corpus. $\times 165$.—Fig. 27. Transsection of young leaf (300 μ high) about 70 μ below tip; *smr*, submarginal row of initials. $\times 165$.—Fig. 28. Transsections of young leaves (about 300 μ high) 140 μ above base; *h*, hair on transitional form; *e*, epidermis of transitional form. $\times 165$.—Fig. 29. Transsection of young

Marginal growth of the primordium.—When a height of 250–300 μ has been attained, marginal growth of a specialized type begins in the adaxial, marginal regions of the primordium. The marginal meristem is initiated by periclinal divisions in the sub-marginal layer and in one row of deeper lying cells (fig. 27–29). These periclinally dividing layers extend along either edge of the primordium from the base to the apical initials, as in *V. rufidulum* (Cross, 1937b). In the region of the midrib these normally behave as follows: Each initial divides first in a plane perpendicular to the margin of the primordium, thus producing an inner and an outer cell (fig. 29, 33). The inner cell divides, and its derivatives may divide repeatedly; augmenting the middle portion of the ridge of tissue which is the young lamina. The outer cell persists as a submarginal initial and soon divides again, this time parallel to the margin of the lamina (fig. 32, 34, 38). Of the two submarginal cells produced by this division, one persists as a submarginal initial, and the other becomes a part of either the adaxial or abaxial subepidermal layer of the lamina. In this manner the lamina is extended by irregularly alternating periclinal and anticlinal divisions of the submarginal row of initials. Three layers of cells are formed which diverge from the submarginal row—i.e., an adaxial subepidermal layer, an abaxial subepidermal layer, and a middle layer (fig. 32, 38). The epidermal layer divides constantly by the insertion of anticlinal walls, but the existence of a specialized marginal row of initials could not be demonstrated.

The behavior of the three layers of cells which diverge from the submarginal initials is generally in conformity with that described for *V. rufidulum*. The adaxial subepidermal layer divides ordinarily by anticlinal walls, only very rarely by periclinal walls. Its derivatives constitute the upper layer of the palisade meristem. The abaxial subepidermal layer divides prevailingly by anticlinal walls, although frequently periclinal walls appear. The middle layer divides both periclinally and anticlinally. It is seen from this that a mechanism is established for the production of a young lamina which may vary considerably in thickness at different levels. The lamina is commonly five layers of cells thick near the margin; however, the appearance of periclinal walls in the middle or one of the subepidermal layers may increase this number. Derivatives of the middle layer form a

meristem which will be involved in the production of the second layer of palisade, the secondary veins, veinlets, and a portion of the spongy mesophyll. Contrary to the condition obtaining in *V. rufidulum*, the adaxial and abaxial halves of the lamina grow at approximately the same rate, thus accounting for the fundamental differences in vernation (plicate in *V. Opulus*, involute in *V. rufidulum*).

While the marginal growth described above apparently is most common, many variations may occur in different leaves or along the margin of the same leaf. A type commonly observed and reported also for *V. rufidulum* is as follows: Strictly periclinal or anticlinal walls are not formed in the submarginal initials, but these are replaced by alternating oblique walls. The initials then become wedge-shaped with only two cutting surfaces. Under these conditions only two layers of cells diverge from each submargin (fig. 36, 37), but a third is usually soon acquired by a periclinal splitting of the abaxial layer (fig. 36). A second, less frequent deviation from the "normal" marginal growth is brought about as follows: The submarginal initial divides parallel to the margin of the lamina, and two submarginal cells are formed, one of which ordinarily should augment either the adaxial or abaxial layers. These two submarginal cells then divide perpendicular to the margin of the lamina (fig. 35), producing two submarginal and two inner cells. However, marginal growth by "twin" submarginal initials is not extensive, and tissues obviously produced by this mechanism were not abundant. It is probable that very soon one of the initials is able to dominate the other, whereupon there is a return to one of the two previously described methods of growth. It seems significant that whatever the behavior of the marginal initials, the cell pattern a short distance removed from the margin remains relatively constant.

Shortly after the initiation of the lamina most of the cells of the adaxial and abaxial layers become highly vacuolate with densely staining protoplasts. Such cells retain their ability to divide. This situation obtains in *V. rufidulum* (Cross, 1937b) as well as in *V. Sargentii*,⁴ and it is probably a generic characteristic.

Marginal growth of the petiolar portion is initiated in much the same manner as that of the midrib,

⁴From observations of slides loaned by Dr. Adriance S. Foster.

leaf (300 μ high) about 110 μ above base; *lo*, lobe. $\times 165$.—Fig. 30. Transsections of margins of young leaves (about 300 μ high) 30 μ above base. $\times 165$.—Fig. 31. Longitudinal section of young leaf with portion of apical meristem; *mr*, young midrib; *p*, petiole; *b*, base. $\times 165$.—Figs. 32, 33, 34, 35, 36, and 37. Transsections at various levels of the margin of a young leaf showing variation and behavior of marginal initials. $\times 325$.—Fig. 38. Transsection of portion of blade of young leaf. $\times 165$.—Fig. 39. Transsection of portion of lamina of young leaf about 8 mm. long; *ve*, veinlet initial; *plm*, plate meristem. $\times 325$.—Fig. 40. Transsection of portion of lamina of mature leaf; *pl*, palisade tissue; *st*, spongy tissue; *gc*, guard cells. $\times 165$.—Fig. 41. Longitudinal section of lobe of young leaf parallel to surface. $\times 165$.—Fig. 42. Transsection of lobe of young leaf showing origin of lamina. $\times 325$.—Fig. 43 and 44. Transsections of margins of laminas of transitional forms showing similarity to foliage leaf. $\times 165$.—Fig. 45. Transsection near middle of partially mature stipular appendage showing tendency to form lamina initials. $\times 325$.—Fig. 46. Transsection of margin of petiole of young leaf showing origin of gland; *g*, glandular out-growth. $\times 165$.—Fig. 47. Diagram of transsection of petiole of completely expanded leaf near juncture with blade, showing marginal position of gland; *gc*, densely staining glandular cells; *ve*, veins. $\times 11$.

although the submarginal rows of initials are formed in a more lateral position (fig. 30). The growth of the petiole closely parallels that of *V. rufidulum* in that a reduced "lamina" or "wing" is produced on each margin (fig. 47). The presence of an abortive lamina on the margins of the petiole suggests that the primitive *Viburnum* leaf might have been sessile and that the petiole has developed through the partial abortion of the lamina of the proximal portion. An originally sessile leaf with three terminal and two or more lateral or basal lobes, by extensive reduction of the lamina in its proximal one-half, could have changed (phylogenetically) into a leaf of the type displayed by *V. Opulus*.

The initiation of the main lobes of the leaf.—The two main lobes of the leaf are initiated when the primordium is 250–300 μ high. They appear as swellings on the margins and regions immediately abaxial to the margins, about 110 μ from the base⁵ of the leaf, at the juncture of the petiole and midrib (fig. 29, 41). Their appearance is correlated with periclinal and anticlinal divisions of the submarginal cells as well as the deeper layers. More rapid growth in the proximal half of the swelling gradually turns the apex of the young lobe upward. A group of subapical initials is soon formed, and the dividing derivatives of these extend the young lobe vertically in the manner described for the midrib (fig. 41). The lamina of the lobe is initiated in the same way and subject to the same variations in marginal growth as the lamina of the midrib. Figure 42 illustrates the origin of a lamina on a lobe through the formation of a submarginal initial with two cutting faces.

Some histogenetic features of the stipular appendages and glands.—A detailed study of the origin and development of the stipular appendages was not made, but a few sections were cut to check the statements of Goebel (that these structures are stalked glands) and Baillon (that they represent lobes of the leaf). Stipular appendages frequently lack glandular tips; moreover, they are usually slightly concupiate like the lobes of the leaf (Lubbock, 1899). From this it would seem that Goebel's view might be open to serious criticism. The primordia of the "stipules" arise after the main lobes of the leaf have appeared, but before the glands are differentiated. This suggests that they represent the lower lobes of a basipetally developing leaf, an hypothesis supported by the presence of a "reduced lamina" on the petiole. Figure 45 was made from a transection of the median portion of a young stipular appendage. A comparison of this figure with figure 42 (young lobe) shows some interesting similarities. A specialized, marginal meristem appears in either edge of the "stipule" comparable in every particular to the marginal meristem in the lobe (fig. 42) and in the main axis of the leaf (fig. 27–30). Provascular tissue appears in the center. Histological evidence thus supports the theory that the stipules are lobes. Further evidence is

⁵ Measuring from the center of the lobe to the juncture of the primordium and the apex of the axis.

afforded by the fact that additional stipular appendages may appear on the margins of the petiole.

Glands arise through the proliferation of the cells at the margins of the petiole or lower lamina (fig. 46); they are not to be considered as major divergences of the foliar axis, but arise secondarily on any portion of the leaf after the major branches (lobes and "stipular appendages") have been formed. They are in no sense homologous with the stipular appendages. On the contrary the evidence here presented strongly supports the view that the stipular appendages are "reduced" lobes, which may or may not produce glands at their tips.

Differentiation of the plate meristem.—The derivatives of the submarginal initials of the lamina diverge inwardly as three layers of cells where they become organized into an extensively dividing plate meristem (Plattenmeristem, Schüeppe, 1926). The divisions are prevalently anticlinal with the following exceptions: An adaxial subepidermal cell near the margin may very infrequently undergo a periclinal division, whereupon the inner derivative augments the internal tissue while the outer enlarges to the size of its adaxial neighbors. The same phenomenon may occur considerably more frequently in the abaxial subepidermal layer. Periclinal divisions were never observed to occur in either of these layers except in the immediate proximity of the margin. The middle layer may divide only anticlinally, or perhaps more frequently it may divide once periclinally, forming a double layer. Including the epidermal layers the plate meristem of *V. Opulus* varies in thickness from five to six or occasionally seven layers of cells as compared to the six to ten layers in *V. rufidulum* (Cross, 1937b).

Secondary veins and veinlets appear early, formed largely from derivatives of the middle layer (fig. 39, 40), for the subepidermal layers do not divide periclinal during the initiation of veins. The subepidermal layers become greatly vacuolated and their contents stain densely, as indicated in figure 38. The maturation of the plate meristem to form the tissues of the adult lamina (fig. 40) will not be described, since no unusual developmental features were noted.

CONCLUSIONS.—The results obtained from this study provide additional evidence that the form and structure of highly specialized cataphylls and foliage leaves are based upon histogenetic processes which may be detected very early in the development of the primordia. No indication of a "transformation process" as described by Goebel could be found in the ontogeny of the bud scale; on the contrary, the primordia of the cataphylls and foliage leaves are committed histogenetically to their respective developmental careers as early as the 80 μ stage. Even earlier than this, a hint of the differences to be expressed later may be obtained by careful staining methods, with which it can be demonstrated that the primordia of the cataphylls stain less densely with Safranin than do the primordia of foliage leaves. This provides some evidence that the stage of histo-

logical divergence is conditioned by an earlier, more subtle phase which is characterized chiefly by physiological differences.

No positive histological evidence concerning the homology of the bud scale could be found. Lubbock's statement that the scale is a modified petiole seems misleading, for the histogenetic processes peculiar to the initiation and development of a petiole do not occur. The present writer accepts the view of Foster (1928) that it is unnecessary to attempt to refer every bud scale to some part or parts of a foliage leaf. As far as the data here presented are concerned, the bud scales of *V. Opulus* lack any such definite homology; their relationship to the foliage leaf is perhaps best expressed by the less rigid concept of "parallel development" (Arber, 1937), by which it is possible to make comparisons without implying homologous relationships. However, the bud scales, transitional forms, and foliage leaves of the related *V. rufidulum* are apparently variants of one basic foliar type and homologous in the accepted sense of the term.

The somewhat unusual histogenetic features of the bud scales of *V. Opulus* should be of interest to students of floral morphology. Comparative histogenetic studies involving the perianths of several gamosepalous and gamopetalous genera should aid in the interpretation of these tubular structures. For instance, the demonstration of vertical meristematic strips (which might be interpreted as the marginal meristems of undiverged foliar organs) below the notches of a gamopetalous corolla would be significant. The importance of data of this type seems to have been recognized in a general way by Cooper (1932) in his studies of the floral organs of *Buginvillaea glabra*, but there is apparently little or no detailed information available on the histogenesis of floral parts other than the carpel.

The extremely variable transitional forms of *V. Opulus* offer interesting possibilities for further study. The present work indicates that the variations in their final form may be conditioned by histogenetic variations evident early in ontogeny. Thus it should be possible to predict at a very early stage many of the characteristics to be expressed in the adult. Such data should add to the significance of the results reported in this paper and others, and the writer hopes to continue histological investigations along this line.

SUMMARY

The morphology and anatomy of the foliar bud of *V. Opulus* are discussed, and certain features pertaining to the histogenesis of the cataphylls, transitional forms, foliage leaves, stipular appendages, and glands are described.

Comparative studies of young cataphylls and foliage leaves show conclusively that histogenetic divergences occur at a very early stage, and staining reactions indicate that these divergent stages are conditioned by even earlier phases which are characterized by physiological differences. No evidence of an ontogenetic metamorphosis could be detected in the development of the primordium of the cataphyll.

Evidence of the homology of the cataphyll to any part of the foliage leaf was not obtained. It is suggested that the relationship of these organs might be expressed in terms of parallelism (Arber, 1937)—i.e., that they have experienced parallel phylogenetic and ontogenetic developmental careers.

Evidence is presented that the stipular appendages are not stalked glands as interpreted by Goebel (1932) but are vestigial lobes of the leaf.

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THE INTERDEPENDENCE OF AUXIN AND SUGAR FOR GROWTH¹

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AT THE beginning of the auxin work on the *Avena* coleoptile, Went (1928) pointed out that in addition to the downward moving auxin there should be some upward moving material necessary for growth. He then called this material *Zellstreckungsmaterial* but later (1935) *food factor*, and gave evidence consistent with the view that it normally moves from the basal to the apical region of the coleoptile. Hence, the basal region should be rich in food factor, the apical region rich in auxin, and the intermediate region fairly rich in both. Having much of both factors, this region should grow more rapidly than the regions above or below it. Went used this consideration to explain the existence of a region of most rapid growth some distance below the tip in the advanced stages of growth of the *Avena* coleoptile.

In his work on geotropism, Dolk (1930) used the food factor concept and gave it certain modifications. Dolk and, later, Went and Thimann (1937) used the term in the sense of a complex of substances.

It is evident from the outset that substances must exist that will fall under the definition of food factor. A suitable method for its determination was found in the "section test" of Bonner (1933). The section test consists of the measurement of elongation of segments of the *Avena* coleoptile immersed in a test solution. To use this test for the present investigation, further analysis of its characteristics and simplification of its technique were carried out.

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This investigation was begun under Prof. F. W. Went at the California Institute of Technology and continued under Prof. K. V. Thimann at Harvard University. The author wishes to take this opportunity to thank Prof. Went and Prof. Thimann for valuable criticism and advice.

METHOD.—Experimental conditions.—*Avena* seeds (Victory oats) were germinated on moist filter paper and grown in an air-conditioned dark-room with occasional red and orange light, at a temperature of 24°C. and a relative humidity of 90 per cent. At first the seedlings were grown in the ordinary glass holders, but later it was found more convenient to grow them on the filter paper where they were germinated, care being taken in arrangement and in supplying water. The seedlings were generally used when about 2-3 cm. long. The auxin, indole-3-acetic acid, was obtained from the Eastman Kodak Co. or from Merck & Co. In general, fresh stocks were made up for each test, but in a few cases, an old stock that had been freshly standardized was used. Both stock and dilutions were made with distilled water. To avoid the complicating effects of salts (see Thimann and Schneider, 1938) and of bacteria, glassware had to be thoroughly cleaned and rinsed. Solutions were not allowed to dry on combs. After brushing and rinsing, the combs were soaked in 70-90 per cent alcohol, then finally rinsed and dried.

Sectioning tool and combs.—The type of sectioning tool described by van der Weij (1932) was chosen. It offers convenience in handling and gives a more reproducible zero value than does the simpler razor-blade cutter previously used in the section test. The guide-holes for the coleoptiles were spaced to match the teeth of combs onto which the coleoptile sections were to be mounted (fig. 1). Fine-toothed combs that would fit into the cover of a 100 mm. Petri dish were chosen. The teeth were ground small enough so that the hollow coleoptile sections could just slide onto them; then every second and third tooth was removed so as to provide sufficient clearance between neighboring sections.

Mounting the sections was made easier by partially inserting the teeth of the combs into the coleoptiles before cutting them off. Besides the advantage of glass combs (used by Bonner) in ease of orientation under the eyepiece micrometer of a microscope (a total magnification of $16\times$ was used), this type of comb has, in conjunction with the van der Weij cutter, additional advantages. These are: greater ease in cutting and handling sections, making it easy to mount serial sections and sets of serial sections in a given order, and preventing them from floating away or rotating (by forcing them onto the wedge-shaped teeth) so that errors caused by measuring different sides in successive readings are avoided.

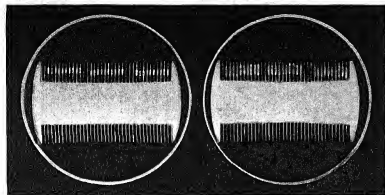


Fig. 1. Coleoptile sections mounted on combs and immersed in solutions in Petri dishes for 90 hours. Left, water controls, about 10 per cent elongation. Right, treated, about 100 per cent elongation with growth in thickness as well. Aug. 25, 1937.

As with Bonner's results for glass combs (1934), sections on these combs grew as well as those not on combs (table 1). It was therefore assumed that no substance affecting auxin, or growth, was liberated from the combs. The sections were put directly into the test solutions after cutting, no preliminary washing being done. Upon immersion, there seemed to be a rapid uptake of water resulting in a large apparent growth rate during the earliest periods of measurement.

TABLE 1. Percentage elongation of sections in 1 mg./l. auxin. Each value is an average from the growth of 30 sections (3, three mm. sections from each of 10 plants).

Date	On combs	Not on combs
Oct. 21	26.9	29.0
	22.1	24.0
Oct. 25	26.2	18.6
	25.7	21.0
	26.9	25.0
Average	25.6	23.5

Level of liquid and oxygen supply.—To obtain well defined readings, it was necessary to have the sections completely covered by the solution during measure-

ment. To insure an adequate oxygen supply, the entire comb and sections could be floated by surface tension and submerged only when the reading was made, but this method could not be used for measurements requiring successive readings because the combs could not be satisfactorily refloated if they had once been submerged. Renewing the solutions, which should renew the oxygen supply, did not increase the growth rate (fig. 2). (The sucrose and auxin plus sucrose curves will be discussed under "Results".) Further, Bonner (1933) reports that stirring by bubbling air through the solution gave no significant increase of section growth and concludes that there is no oxygen deficiency. With all this evidence pointing against an oxygen deficiency, the sections were left submerged.

TABLE 2. Percentage elongation of sections in various volumes of auxin or of water for 33 hours. The growths in auxin are averages of tests done on Sept. 22 and Oct. 2; water, Oct. 6 and Oct. 8. Each test was the average of 30-60 sections (3, three mm. sections per plant).

Cc. per 30 sections	0.4	2.0	10	40
Auxin, 2.5 mg./l. . .	32	37	40	40
Distilled water . . .	12	10	9	9

Volume of solution.—The volume of solution into which the sections were placed was found to have an effect on subsequent growth, the direction of this effect depending on whether the solution were auxin or water (table 2). For the auxin treatment large volumes tended to increase the subsequent growth, whereas for the water treatment large volumes tended to decrease it. Table 2 is in good agreement with

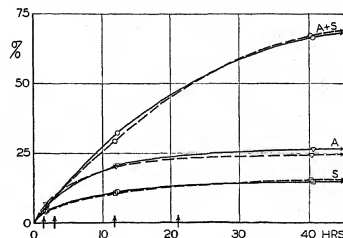


Fig. 2. Growth of sections if solutions are renewed (broken lines) or not renewed (solid lines) in: sucrose (S), auxin (A), and auxin plus sucrose (A + S). (There were no water controls.) Concentrations are: auxin 1 mg./l.; sucrose 1 per cent. Arrows indicate the times at which the sections were rinsed in distilled water for 2-3 minutes and placed in a fresh sample of the original solution. Each curve is from an average of 120 sections (4, three mm. sections from each of 30 plants). Growth in per cent, time in hours. Aug. 26, 1937.

the data of Jost and Reiss (1937) for varying volumes of auxin solution with 1 cm. sections. It is to be seen that the use of large volumes has the technical advantage of low response in controls and of high response in treatment. From table 2, it appears that 40 cc. per set of 30-40 sections is a satisfactory volume to use, and it was adopted as standard volume.

Lower response to the smaller volumes of auxin is probably occasioned by a partially lowered auxin concentration (concentration lowered because the sections use up or destroy auxin), while lower response to larger volumes of water is probably occasioned by an increased, irrecoverable loss of some factor, perhaps auxin, into the water.

On this account, the effect on growth of frequent renewal of solution was tested. From the results, shown in figure 2, and from the results of comparable parts of figures 8 and 9, it is clear that growth even of sections stimulated to an extreme growth rate is not affected by the mere renewal of solutions. Hence, if something can be lost from the tissues, the concentration of it built up in 40 cc. is too low for the substance to be recoverable by the sections.

Relation of growth to auxin concentration.—It was found in the *Avena* test (Schneider and Went, 1938) that the length of the interval between the first decapitation and putting on of the auxin-agar block (abbreviated IDP) was of great importance both for sensitivity of the plants to auxin and for the range of concentrations over which curvature was proportional to concentration. Similar effects of increasing IDP on comparable straight growth measurements were found, the results being shown in figure 3.

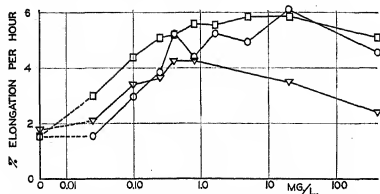


Fig. 3. Percentage elongation per hour of decapitated *Avena* seedlings when agar and a series of concentrations of auxin in agar are symmetrically applied to the cut surface. Circles for 3-5 minute IDP, triangles for 100 minute IDP, and squares for 200 minute IDP. Averages from tests of Aug.-Oct., 1937.

These tests were performed by marking a 10-12 mm. zone below the cut surface of a decapitated *Avena* seedling from which the primary leaf had been removed and measuring elongation over a period of 90-120 minutes. The auxin was symmetrically applied in 2.1 per cent agar blocks prepared by the method of soaking the agar in a large volume of auxin solution made up in distilled water. A second decapitation was made just before applying the

auxin-agar. The IDP, 5, 100, and 200 minutes, show responses parallel to those found in the *Avena* curvature test by Schneider and Went (1938) under similar conditions and methods. Thus, with increasing IDP, the maximum growth rate passes through a minimum value, and the range of auxin concentrations over which growth is a function of the applied concentration is shifted toward lower values. This explains, at least in part, the discrepancy found by Thimann and Bonner (1933) between analogous straight growth measurements and the *Avena* curvature test; they quote different IDP for the two kinds of measurements. The (almost) maximal responses attained with each IDP (fig. 3) for the same concentration ranges as gave maximum angles in the *Avena* curvature test indicate that the lateral transport of du Buy and Nuernbergk (1930) has only a secondary effect on the maximum angle; that is to say, from figure 3 it is clear that lateral transport can only reduce the maximum angle whose occurrence is largely the result of a maximum growth rate.

It is a curious fact that the growth of the agar controls of figure 3 is independent of the IDP. This is hardly what would be predicted from experiments on "regeneration of the physiological tip" (for discussion of "regeneration," see Dolk, 1930).

From similar comparisons of IDP for the section test (fig. 4), it is clear that any IDP gives good results and that, in a gross manner, these results are about the same for all IDP. However, it is advisable for a given set of measurements to use the same IDP for all treatments. Figure 4 and similar tests are at variance in certain details; only those characteristics that are reproducible will be discussed:

1. For short time measurements (1-2 hours) with all IDP there is: (a) a range of concentrations (roughly 0.01-0.2 mg./l.) in which growth is a function of the auxin concentration; (b) a higher range of concentrations (roughly 0.2-5 mg./l.) over which growth is independent of concentration; (c) a still higher range of concentrations (roughly 20-100 mg./l.) giving a further acceleration of growth; and (d) finally an inhibitory range (above 100 mg./l.) giving about the same growth as water controls or even less.

2. For long time measurements (24-100 hours) with all IDP: (a) growth is approximately proportional to the logarithm of the auxin concentration up to 1-10 mg./l. where an optimum is reached; (b) the inhibitory concentration is one or more logarithmic units lower than for short time readings.

Optimal auxin concentration.—For such measurements as those where other factors were added it was necessary to establish the optimal auxin concentration. Optimal values obtained in tests by the author, together with others by Dr. Thimann, were: 1, 2, 2, 2, 2, 9, 10, 10, 10, 30 mg./l., giving an average of 7 mg./l. Occasionally still wider divergence was found, but this occurred in tests giving such erratic responses as to make the results unreliable. In most tests the probable error for 10 sections was of the order of 5 per cent of the increment.

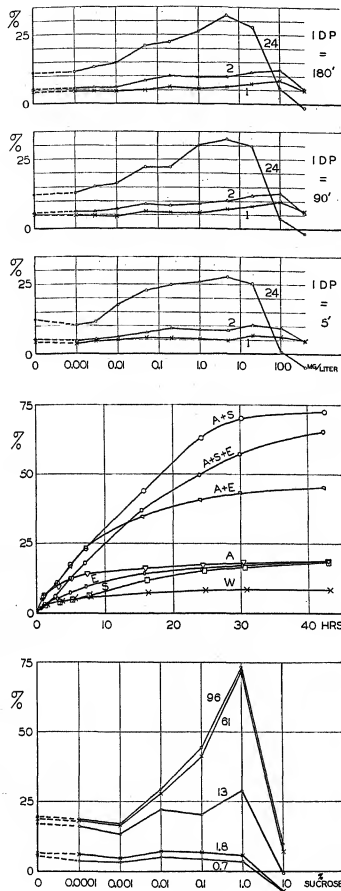


Fig. 4 (above). Growth of sections at 1, 2, and 24 hours in increasing auxin concentrations for 5, 90, and 180 minute IDP. Each concentration is from an average of 30 sections (3, three mm. sections from each of 10 plants) except for water controls which are from 60 sections. For discussion, see text. Sept. 21, 1937.

Fig. 5 (center). Growth of sections in various solutions: distilled water (W), sucrose (S), seed extract (E), auxin (A), auxin plus extract (A+E), auxin plus sucrose (A+S), auxin plus sucrose plus extract (A+S+E). Concentrations are: auxin, 1 mg./l.; sucrose, 2 per cent; seed extract, approximately 1 seed/10 cc. Each

Variations in section growth have been reported previously by Bonner (1933) and by Jost and Reiss (1937).

In further experiments with "food factor" treatment, 1 mg./l. was almost always the optimal auxin concentration, and since this was near the average optimum for treatment with auxin alone (the relation of growth to auxin concentration being logarithmic), it was chosen as the standard optimal auxin concentration.

RESULTS.—Food factor effect of seed extract.—In preliminary tests it was found that sections beginning at 5-6 mm. below the tip of the coleoptile as described above and placed in auxin of optimal concentration for 24 hours grew about twice as much as water controls (fig. 5). Adding food factor should further increase this growth. Since the ultimate source of the food factor must be the endosperm of the seedling, a cold water extract of seeds of three-day-old seedlings was prepared and tested for food factor content.

Some of the preliminary experiments indicated that auxin added to the extract was being destroyed by enzymes. Thereafter the solutions were boiled, which besides destroying enzymes, delayed bacterial growth.

Addition of the seed extract considerably increased growth either in the presence or absence of added auxin. The optimal concentration corresponded approximately with one seed per ten cc. of water. Figure 5 shows that this optimal concentration without auxin gave about the same growth as optimal auxin.

Combination of optimal auxin plus optimal seed extract gave about four times as great growth as either alone. Hence it is clear that the extract had a growth promoting effect different from that of auxin and that this effect was greatest in the presence of auxin. Since sugar is probably the major constituent of the seed extract, the action of sugar was then studied in similar tests.

Food factor effect of sucrose.—An optimal sucrose concentration (determination of this optimal value is given below) gave the same growth as the optimal seed extract concentration. Comparison showed that the combination of optimal sucrose plus optimal auxin gave even more growth than seed extract plus auxin (fig. 5).

To test whether the growth effect of sucrose and extract were identical, tests were run with the three materials—auxin, seed extract, and sucrose—in com-

parison. The growth effect of sucrose and extract were identical, tests were run with the three materials—auxin, seed extract, and sucrose—in comparison. The growth effect of sucrose and extract were identical, tests were run with the three materials—auxin, seed extract, and sucrose—in comparison.

Figure 6 (below) shows the growth of sections in 1 mg./l. auxin plus increasing sucrose concentrations at 0.7, 1.3, 1.8, 6.1, and 9.6 hours after cutting the sections. The values for each concentration are from an average of 30 sections (3, three mm. sections from each of 10 plants), except the water control which is from an average of 60 sections. Oct. 11, 1937.

bination in optimal concentrations. If the effect of the seed extract were different than the effect of the sucrose, then the combination of the two with auxin should give a combined effect greater than either extract plus auxin or sucrose plus auxin. Actually the combination of auxin, seed extract, and sucrose gave the same effect (not quite as large) as auxin plus sucrose (fig. 5). Hence, the effect of the seed extract is the same as that of sucrose and is probably due to sugar contained in the germinated seeds.

Other sugars as food factor.—Comparisons were made for the food factor effects of a few sugars and of mannitol (table 3). The substances were extracted with ether according to the method of Thimann (see Went and Thimann, 1937, p. 189). Each of the sugars gave a strong effect, but mannitol gave such a

That maltose gave a lower response than the other sugars in this test is not necessarily significant because it was an impure sample. However, in a previous test with Pfanzstielh sugars, maltose also gave a much lower response than sucrose, levulose, or dextrose, all of which in that test gave about equal results at concentrations of 1–2 per cent. Nor can conclusions as to the concentration of maltose in the seed extract, of which the sugar is thought to be mostly maltose, be drawn from the relative responses to the seed extract and to maltose (fig. 5 and table 3), because the seed extract probably contained at least some other sugars and very likely some inhibiting substances.

It is clear that the food factor effect of the seed extract and of sucrose, studied above, is a sugar

TABLE 3. Food factor effect, in the presence of 1 mg./l. auxin, of various sugars, and of mannitol. The values given are average percentage elongations from 30 sections (10 plants). Aug. 6, 1937.

Time of reading	22 hours				47 hours			
	0	0.2	1.0	5.0	0	0.2	1.0	5.0
Sucrose		25.5	38.6	24.6		34.9	50.8	46.2
Levulose		23.7	32.5	9.8		31.0	41.7	20.7
Dextrose	12.2	25.8	30.0	2.1	12.5	34.5	40.7	12.4
Maltose		22.2	27.1	12.0		25.2 ^a	23.1 ^a	5.2 ^a
Mannitol		10.2	9.2	-1.2		11.7	14.8	0.5

^a Heavily infected.

minute response that it may have been caused by sugar impurities in the sample, or more likely, since it became appreciable only late in the test, by sugar produced from the mannitol by bacteria. Indeed, improved tests in another connection (see Thimann and Schneider, 1938) show no acceleration of growth by mannitol.

effect, for other sugars have been shown to give it, whereas a closely related non-sugar, mannitol, did not.

Simultaneous limitation of growth by auxin and by sugar.—Figure 5 shows that in the presence of optimal auxin, growth can be limited by sugar. From figure 5 it also appears that growth can be limited by sugar while it is simultaneously limited by auxin,

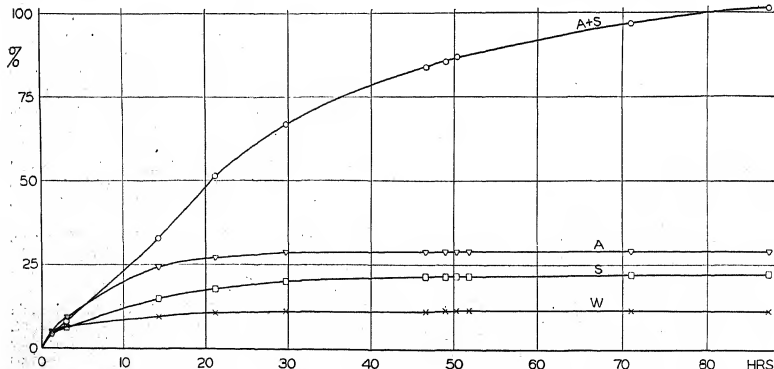


Fig. 7. Growth of sections in: water (W), sucrose (S), auxin (A) and auxin plus sucrose (A + S). Concentrations are: auxin, 1 mg./l.; sucrose, 1 per cent. Curves W and A + S are averages from 90 sections (30 plants); curves S and A are averages from 120 sections (40 plants). Aug. 26, 1937.

for the growth of sections containing only their residual auxin is promoted by the addition of sugar alone. This data is in good agreement with the less extensive work of Bonner (1934) in which he used 1 per cent fructose (levulose) on sections of *Avena* coleoptiles in a manner similar to that of these experiments. His data also show that sections in 1 per cent levulose grew more than in water and less than in 10 unit auxin² and that the combination of 1 per cent levulose and 10 unit auxin gave a greater growth than either alone.

To test whether sugar can limit growth simultaneously with auxin, it is necessary to have a sugar source that is quite free of auxin. The seed extract

auxin-free sugar alone increases growth (curves *S* and *W*). This test has been repeated many times. There was some variation in the absolute values for the *S* curve and in the relative final growths for the *A* and *S* curves (compare figs. 7, 8, tables 4, 5), but the sugar curve was always higher than the water curve.

Tables 4 and 5 summarize more detailed tests on different concentrations of auxin and sucrose separately and together. It is evident from them, also, that sucrose may have a growth effect of its own even though auxin be limiting growth at the same time. Further, it is remarkable that supplying a second limiting factor, sugar, never shifted the optimal auxin concentration toward higher values, but

TABLE 4. Percentage of growth of the 1st, 2nd, 3rd, and 4th serial sections (3 mm. long), beginning at 6 mm. below the tip of the coleoptile, and their average growths for various auxin and sucrose concentrations separately and together at 48 hours. Ten plants were used for each treatment except the water control, where 20 were used. Aug. 4, 1937. See also table 5.

Auxin mg./l.	Section	Sucrose concentration in per cent			
		0	0.5	1.0	2.0
0	1st	11	20	21	19
	2nd	9	16	18	20
	3rd	6	13	17	12
	4th	6	11	18	13
1	1st	28	73	86	85
	2nd	24	67	77	60
	3rd	23	55	70	56
	4th	21	51	58	47
3	1st	31	61	65	79
	2nd	22	52	51	71
	3rd	19	45	52	61
	4th	20	41	47	54
10	1st	28	62	65	72
	2nd	21	48	45	46
	3rd	18	46	46	57
	4th	21	42	34	46

that had been used was not well suited for this purpose because it sometimes contained auxin and always contained much extraneous matter. Pure sugars, on the other hand, can be easily obtained. To get sugar that was free of auxin, a sucrose syrup was extracted with ether according to the method of Thimann (see Went and Thimann, 1937, p. 189). The extraction was repeated three times to remove even traces of auxin and incidentally of inhibiting substances occurring in sugars.

To determine the optimal sucrose concentration, a series of concentrations was tested in the presence of optimal auxin (fig. 6). The optimal sucrose concentration varied from 1-2 per cent, 1 per cent being adopted as optimum. Using this value, the growth measurements of figure 7 were obtained. This figure shows that not only does sugar increase the growth effect of auxin (curves *A* and *A+S*) but also that

tended to shift it toward lower values. Tables 4 and 5 are examples of the constancy of the optimal auxin concentration of 1 mg./l. in the presence of optimal sucrose (which compares with the average 7 mg./l. for auxin alone).

Analysis of the interdependence of auxin and sugar as limiting factors for growth.—It has been postulated by Went (1928) that "ohne Wuchsstoff, kein Wachstum"—i.e., growth can occur only in the presence of auxin. However, the above experiments indicate that growth is increased by supplying sugar alone. This raises the question whether such increase of growth by sugar is due to auxin already present in the sections and conversely whether growth produced by auxin is due to food factor or sugar already present.

To answer this question, it was desired to have sections that would be free of food factor and of auxin since such sections would be expected to grow only if both sugar and auxin were then added. However, because of bound auxin (for discussion, see Went and Thimann, 1937, p. 132), the sections could

² Bonner used a partially purified *Rhizopus* culture extract; 10 unit solution equals approximately 0.1 mg./l. indole-3-acetic acid.

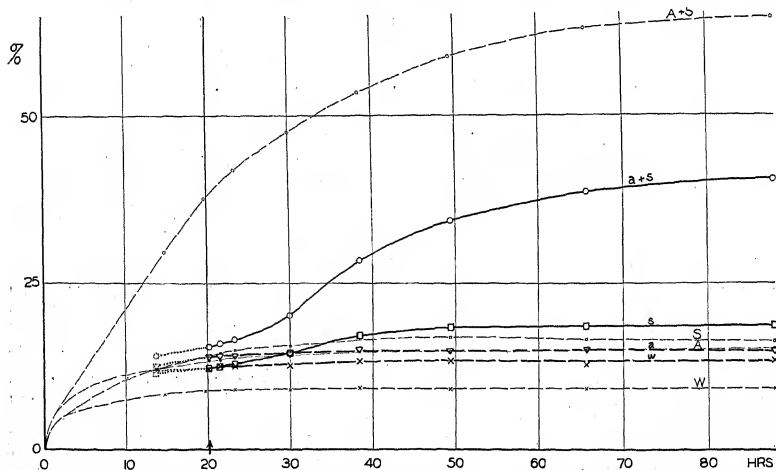
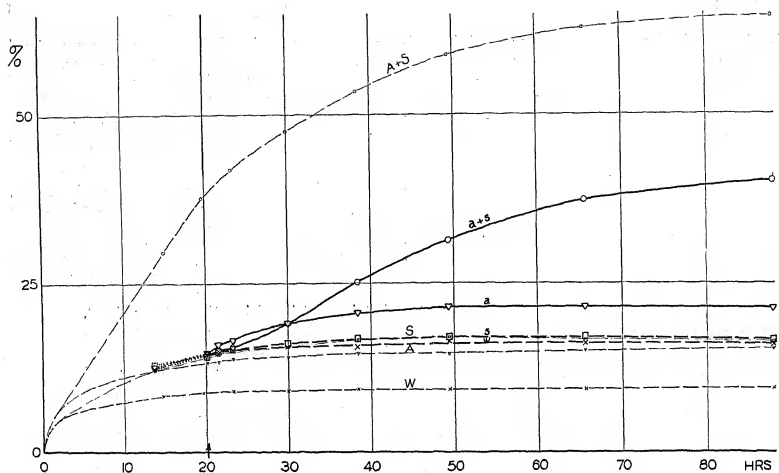


Fig. 8 (above). Response of auxin-deficient sections to treatment (heavy solid line curves) with auxin (*a*) and with auxin plus sucrose (*a+s*). Auxin-deficient controls (heavy broken line curves) were treated with water (*w*) and sucrose (*s*). The sections were allowed to become almost auxin deficient by growing in sucrose for 20 hours. Then they were rinsed in distilled water and changed to the new solutions (at the time of the arrow). The fine, broken line curves (*W*, *A*, *S*, and *A+S*) show the growth of additional controls; they are a repetition of the treatments shown in figure 6 and were kept in the same solutions from the beginning of the experiment. Concentrations are: auxin, 1 mg./l.; sucrose, 1 per cent. Sept. 1, 1937.

Fig. 9 (below). Response of sugar-deficient sections to treatment (heavy solid line curves) with sucrose (*s*) and auxin plus sucrose (*a+s*). Sugar-deficient controls (heavy broken line curves) were treated with water (*w*) and auxin (*a*). Method as for figure 8. Additional controls (fine broken line curves) are identical to those of figure 8. Data for figures 8 and 9 come from the same experiment. Sept. 1, 1937.

not normally be expected to use up all their auxin in growth or give it off into the solution. Preliminary attempts to get rid of auxin by putting the sections into buffer of low pH, which should convert bound auxin to the active form and cause it to be eventually used up in growth, were made. Such experiments were consistent with the contention that both auxin and sugar were necessary for growth, but they were inadequate because some auxin remained in the sections.

It was then found that sections could be depleted of auxin by allowing them to grow in sugar solution and, conversely, that they could be depleted of food factor by allowing them to grow in auxin. Hence it was possible to get sections depleted of either food factor or of auxin but not simultaneously depleted of

(*w*), auxin (*a*), sucrose (*s*), or auxin plus sucrose (*a+s*), they responded moderately to auxin and vigorously to auxin plus sucrose but not at all to water or to sucrose.

Similarly, sections almost depleted of food factor responded moderately to sucrose and vigorously to auxin plus sucrose but not at all to water or to auxin (fig. 9).

Thus for both cases there was a definite growth response to a renewed supply of the deficient substance alone. This response was of limited magnitude, probably because the storage of the other factor was too small to give an extensive growth under these conditions. This conclusion is borne out by the curves for addition of the deficient and the non-deficient substances together (*a+s* curves of figs. 8, 9). They

TABLE 5. *Explanation as in table 4. July 31, 1937.*

Auxin mg./l.	Section	Sucrose concentration in per cent		
		0	2	4
0	1st	19	35	20
	2nd	15	36	21
	3rd	11 14	25 29	12 16
	4th	11	19	9
1	1st	26	86	64
	2nd	22	77	54
	3rd	19 20	83	46 49
	4th	13	77	31
3	1st	33	93	72
	2nd	31	71	47
	3rd	22 26	62	46 49
	4th	18	35	31
9	1st	38	85	45
	2nd	26	75	38
	3rd	29 30	65	44 41
	4th	26	49	35
30	1st	29	57	40
	2nd	26	47	33
	3rd	21 24	56	28 30
	4th	20	54	20

both. To stimulate a renewed growth of good magnitude in such sections, it was necessary to supply the deficient substance as soon as the sections became sufficiently free of it to suit the purposes of the test. (With increasing time after cutting the sections, there is a decrease in the maximum possible growth rate, for which see discussion of table 6.) The possibility of getting sections free of auxin by allowing them to grow in sugar and of using up practically all of their auxin thereby is a limiting case of the view of Bonner and Thimann (1936) that the amount of growth produced is proportional to the amount of auxin inactivated. Indeed, the procedure for getting auxin-free sections came from a consideration of this view. As will be seen below, the case for sugar and its deficiency turned out to be analogous to that for auxin and its deficiency.

If separate groups of sections thus almost depleted of auxin (fig. 8) were supplied with distilled water

show a gradually increasing growth rate that finally approaches the rate of controls placed in auxin plus sucrose from the outset.

Also in other measurements of recovery from deficiency similar to those of figures 8 and 9, but allowing different periods for the deficiency to develop, the maximum growth rate attained upon recovery was about equal to the growth rate, at the same time, of controls placed in auxin plus sucrose from the beginning of the experiment (table 6). From table 6, it appears that the maximum growth rate, and therefore the total growth possible, is decreased by some process that progresses rapidly at first and then more slowly. This process is independent of the growth rate, for reduction of the capacity for growth goes on in deficient sections, which are not growing, as well as in the growing, auxin-plus-sucrose controls of figures 8 and 9 and of table 6. Also, it is largely independent of the presence of either auxin or sugar, for,

as seen in table 6, its effect on the amount of recovery from either kind of deficiency is about the same. This process may be tentatively considered as a decrease in the amount of a third "limiting" factor.

Besides the amount of recovery possible, another process can be studied from figures 8 and 9—namely, the rate of recovery. In figure 8 it may be seen that the addition of auxin to auxin-deficient sections causes a rapid acceleration of growth. On the other hand, figure 9 shows that the addition of sucrose to food factor-deficient sections causes only a slow acceleration of growth. Similar results are obtained if the deficiency is allowed to develop during a longer or a shorter time than in figures 8 and 9.

This slow response of auxin- or sugar-deficient sections to a supply of auxin plus sucrose can hardly

vantage, for in all three of these treatments the sections go into a new solution of osmotic concentration equal to or greater than that of the one from which they have just come. They show a typical sigmoid growth curve. Thus, this small observed difference in recovery from these two kinds of deficiency seems to be controlled by the method of doing the experiment and serves further only to demonstrate the close similarity of recovery from the two types of deficiency.

A demonstration of the close similarity of auxin and sugar as interdependent factors for growth is given in table 7. Table 7 shows that, for the two 10-hour periods 4-14 and 14-24 hours, for concentrations where growth is a function of concentration, there is an increase in growth rate for an increase of either auxin or sugar.

TABLE 6. Recovery from deficiency of auxin or of sugar. The values given are in each case the maximum growth rate attained after supplying 1 mg./l. auxin plus 1 per cent sucrose. For comparison, the growth rate of controls at the same time (about 14-18 hours after application of the auxin plus sucrose, the interval increasing with time allowed for the deficiency to develop) is given.

Date of experiment	Oct. 4	Sept. 1		Aug. 20
Hours for deficiency to develop	4.1	20.2	30.0	58.0 ^a
Growth rate in per cent per hour for auxin plus sucrose controls	1.55	0.73	0.32	—
Maximum growth rate in per cent per hour for recovery in auxin plus sucrose, from:				
Sugar deficiency	1.56	0.71	0.50	0.05
Auxin deficiency	1.69	0.96	0.39	0.03

^a Conditions not strictly comparable.

be occasioned by slow entry. From the *Avena* curvature test and other sources, it is well known that auxin becomes readily available for growth, and now it has been shown by Sweeney and Thimann (1938) that, for some processes at least, sugar becomes about as readily available to the tissues. They measured the effects of sugars on protoplasmic streaming in the epidermal cells of the *Avena* coleoptile and found a response within a few minutes, the length of the time interval varying with different sugars.

The rapid response of the auxin-deficient sections to auxin alone can be explained by considering the difference between the internal and external osmotic pressures, the force causing growth. (For consideration of the osmotic effect of an external solution on growth, see Thimann and Schneider, 1938.)

In the case of the almost immediate response of auxin-deficient sections to auxin alone, the osmotic effect and the auxin effect are working in the same direction, for the sections, having just come from an extended exposure to sucrose solution, probably have an increased osmotic concentration inside and are put into an auxin solution having practically no osmotic pressure. None of the other three treatments for recovery from deficiency— $a+s$ for auxin deficiency, and a and $a+s$ for sucrose deficiency—have this ad-

DISCUSSION AND CONCLUSIONS.—Since sugar can stimulate growth in sections of *Avena* coleoptiles and is obtainable from a basal part (the endosperm) of the *Avena* seedling, it is consistent with the definition of Went to call sugar a food factor. Because sugar has, in conjunction with auxin, such pronounced effects on growth, it must be a leading component of the food factor complex.

Further, sugar may limit growth at the same time that auxin does, since sections of coleoptiles can be stimulated to grow by the addition of sugar alone even though their auxin supply be far below the optimum.

The same conclusions may be drawn from the results of Flry (1932) on *Helianthus*. She found that growth was stimulated in decapitated seedlings when auxin was supplied to the cut surface and that it was stimulated about an equal amount if sugar was supplied instead. If both auxin and sugar were given, growth was stimulated more than by either alone—in fact, almost as much as if the tip, the natural source of both auxin and food factor, had been replaced: Here also sugar has been a food factor and has been limiting growth simultaneously with auxin.

Additional evidence consistent with this "modified limiting factor" view is the tendency for the optimal

auxin concentration for section growth to tend to shift toward lower values when a second growth factor, sugar, is supplied. Not in a single case did it shift toward higher values as the conception of absolute limiting factors would require.

Dolk (1930) arrived very early at the conclusion that the relation between auxin and food factor for growth does not follow the ideal Blackman limiting factor curve (cf. Went and Thimann, 1937, fig. 35). His conclusions are based on growth measurements of geotropic responses. They serve to summarize the relationship between food factor and auxin for the direct evidence obtained here by use of the section test as well as for his own indirect evidence. He states, in part, "I propose, therefore, to follow Went's description of the growth process, as a basis for discussion, but with the reservation that the two

tensity of light have interdependent effects. (See also Romell, 1926.) Thus the theory of limiting factors was developed for the two processes—photosynthesis and growth (Blackman, 1905)—and has on further analysis held for neither of them.

The development of the "absolute limiting factor" theory depends essentially on the process's being controlled by the rate of supply of various factors (of which Blackman, 1905, lists, exclusive of "unessential" substances such as enzymes, five for photosynthesis). If we think, instead, of intensity or concentration in the field of the reaction (which is some function of the rate of supply), then we are able to apply the mass action law of physical chemistry—i.e., that the rate of a chemical reaction is proportional to the product of the active masses of the reacting substances.

TABLE 7. Percentage elongation at 4, 14, and 24 hours for sections in serial auxin and sucrose concentrations separately and together. Values given are averages from 80, three mm. sections (from 20 plants) except where marked * where half that number were used. Dec. 5, 1937.

Auxin concentration in mg./l.	Sucrose concentration in per cent				
	0	0.01	0.04	0.16	0.64
0	6.6	8.0	*	*	7.2
	9.4	12.0	8.9	8.8	13.8
	10.6	13.1	13.6	16.3	16.1
0.01	*				
	9.7	7.9	7.3	9.3	12.0
	13.7	11.5	11.5	16.0	18.8
0.04	14.6	13.6	13.7	19.8	22.2
	*				
	15.0	12.8	13.3	11.5	12.5
0.16	21.0	21.4	23.4	23.8	29.8
	23.0	24.0	26.6	29.9	39.2
	*				
0.64	15.1	11.7	12.4	13.2	13.7
	21.0	23.0	23.5	29.2	35.8
	22.6	26.3	28.3	39.1	48.9
	13.9	13.7	12.5	15.2	15.7
	24.4	25.1	24.5	33.2	39.9
	26.3	29.0	28.9	42.5	53.7

factors exert only a relative and not an absolute limitation on growth. In the whole coleoptile, then, the growth depends on both factors, but their influence is quantitatively different. Thus, in the tip, a change in the Z.S.M. factor (Zellstreckungsmaterial or food factor) will cause a marked difference in growth, while a change in the amount of growth substance will have only a rather slight effect. In the base the reverse is the case." (Translation by Dolk-Hoek and Thimann, 1936.)

The finding that auxin and sugar have interdependent effects on growth is a second case in which it has seemed that the Blackman theory applied, but on closer analysis has not held. It is similar to the finding of Harder (1921) that in photosynthesis the application of the simple limiting factor theory is invalidated because the concentration of CO_2 and in-

Since growth is proportional to the logarithm of auxin concentration and to the logarithm of sugar concentration, this product law—i.e., the following equation—should be expected to hold:

$$[\log(A)] [\log(S)] / G = K.$$

In this formula, (A) equals the concentration of applied auxin, (S) equals the concentration of applied sucrose, G equals growth rate, and K equals a constant whose value is determined by other factors.*

* That, at any given time, these other factors will be independent of the treatments has already been shown above where the maximum growth rate attainable decreased in the same manner for conditions of optimal or minimal growth and in presence or absence of either auxin or sugar. Hence, although K changes with the conditions, it changes to the same extent for all treatments.

Values of K for two successive 10-hour periods are given in table 8. They are sufficiently constant to show that the equation above serves as a first approximation for describing the interrelations of these factors for the growth process. The apparent non-applicability of this formula to the growth rate of the first 4 hours calls for special mention.⁴

In the early hours of the section test, the sections grow at about the same rate in all but low auxin concentrations so that the above formula does not apply. Similarly in the *Avena* curvature test, which depends also on short time measurements, such a formula does not appear to apply because the relationship of auxin concentration to curvature can give the appearance of the ideal Blackman curve (cf. Went, 1928, or Went and Thimann, 1937, fig. 19). This measure of growth rate has, indeed, been the chief reason for continuing to believe that the Blackman theory applied for the growth process. However, not only has it recently been shown (Schneider and Went, 1938) that the interval between decapitation and application of the auxin shifts the point at which the curve of proportionality crosses the axis, but now it has also been

show a relationship that can be more easily interpreted as logarithmic than as that of a Blackman system.

The course of growth, with time, in these three types of measurement, *Avena* curvature test, straight growth of decapitated *Avena* seedlings, straight growth of *Avena* coleoptile sections, and a fourth type, the split stem test with *Pisum* (for results in the "pea test" to be compared here, see van Overbeek and Went, 1937), show certain general similarities. For each method the growth rate, during some early hours of the test, is a function of auxin concentration up to some maximum growth concentration. In the section test and pea test, where the time interval over which measurements are taken may be extended to some later hours (without secondary complications) this maximum growth concentration increases until it gives an optimal growth concentration. This difference in response in the early and late hours of these growth tests is explainable if we postulate that the growth rate is limited by the capacity of the growth system.

If this postulate holds, then we describe growth in all these tests for both early and late hours by the

TABLE 8. Values of K for the intervals 4-14 and 14-24 hours calculated from data of table 7 according to the formula presented above.

Auxin concentration in mg./1000 l.	Sucrose concentration in thousandths of one per cent							
	4-14 hours				14-24 hours			
	10	40	160	640	10	40	160	640
10	0.28	0.38	0.47	0.41	0.49	0.73	0.58	0.83
40	0.19	0.25	0.29	0.25	0.62	0.80	0.58	0.46
160	0.19	0.32	0.30	0.28	0.67	0.73	0.49	0.47
640	0.25	0.36	0.34	0.33	0.72	0.98	0.67	0.57

shown (Thimann and Schneider, 1938) that the concentration of agar, besides profoundly affecting this point of transection, also changes the form of the curve. Thus it is by no means clear that theoretical significance need be attached to the results for one arbitrary set of conditions.

Another growth analysis, the direct growth measurement of decapitated *Avena* seedlings by Thimann and Bonner (1933) and the confirming ones of Scheer (1937), as well as similar ones by the author (fig. 3)

⁴ Incidentally, if this formula can be generally applied, it supports a contention already in the literature. Schneider and Went (1938) have shown that the sensitivity of coleoptiles to auxin in the *Avena* curvature test increases with time after the first decapitation. It has been shown above that the same is true for comparable straight growth measurements. As a partial explanation of this increase in sensitivity, Schneider and Went postulated that the food factor content increases after decapitation. This postulate is not compatible with the classical Blackman theory because it requires that increase of a second factor (food factor) increase the rate of a process (growth) over the range where the rate of that process is proportional to the concentration of another factor (auxin). However, it is supported by the "modified limiting factor system" developed above.

above formula but keep in mind that G has an upper limit, the limit being set perhaps by the capacity of an enzyme system—e.g., that of the cyanide inhibited reaction of Bonner and Thimann (1935). This limit, then, could be approached by increasing auxin, or sugar, or other factors. For the first few hours in the section test, these factors are all present in high amount so that by increasing one of them, auxin for example, we soon reach the upper limit of G . After some longer time; the supply of other factors has been partially diminished (concentration lowered), and we get increased growth over a longer range of auxin concentrations, each concentration, however, being less effective than during the earlier periods. The same follows for the pea test from the results given by van Overbeek and Went (1937). It might be expected that sugar should similarly increase the growth rate in the early hours. That this does not follow in practice for the early hours of the section test may be partly explained by osmotic hindrance to growth that is introduced by the sugar itself (consider in this connection the discussion of figures 8 and 9) and partly by the high content of food factor in the sections. That the food factor storage can be

high is well demonstrated by the "deseeded *Avena* test" of Skoog (1937) where large curvatures are obtained even though the seedlings have had the endosperm removed for 12-18 hours and have grown 100 per cent or more during that time. The effect of sugar was therefore tried on the sensitive *Avena* curvature test to see if an early sugar effect could be detected.

For this purpose, sucrose, fructose, and maltose were tried, with a concentration of 1 per cent appearing to be most effective. To avoid the differential uptake of auxin encountered in soaking agar blocks in the desired solutions (see Thimann and Schneider, 1938), the auxin-agar blocks and auxin-agar-plus-sugar blocks were prepared by mixing equal volumes of melted 3 per cent agar with solutions of double the final concentrations desired; this gave 1.5 per cent agar blocks. Only short IDP seemed to give any effect at all. The only auxin concentration that seemed to give an increased curvature in the presence of sugar was 0.2 mg/l., somewhat below the maximum angle concentration for these conditions. But even there the effect was too small to be reliable; the increase from the averages of 28 test rows being 1.8° on an angle of 30°. Here again, as for the section test, the unfavorable osmotic relations and an abundance of stored food factor probably obscure the sugar effect.

However, that food factor and auxin do have interdependent effects on growth even during conditions comparable to these early hours has already been concluded by Dolk for intact plants as quoted above. It is therefore consistent with the facts known at the present to conclude that growth, for both the early and late hours of the tests considered above, is simultaneously a function of the concentrations of several

factors. If, now, it be further postulated that the rate of the growth process has an upper limit set by the capacity of the system, then the above formula serves as a first approximation for the description of this interdependent relationship for two of these factors, auxin and sugar.

SUMMARY

The technique of the *Avena* coleoptile "section test" of Bonner has been investigated and modified.

An extract of the endosperm of the *Avena* seedling has been shown to promote growth of sections particularly in the presence of auxin. This effect is due to the sugar content of the extract. Sugar is therefore a major component of the food factor complex postulated by Went.

Results of treating auxin-deficient sections with auxin have confirmed the postulate of Went that auxin is necessary for growth. Similar tests—i.e., treating sugar-deficient sections with sugar—show that sugar is also necessary for growth.

It was found that under certain conditions, the effects of auxin and sugar on growth are interdependent in such a manner that, for sub-optimal concentrations, an increase in concentration of either one gives an increase in growth rate. The magnitude of this increase is formulated as proportional to the product of the logarithms of the concentrations.

By postulating an upper limit to the growth rate, set by the capacity of the growth system, it is consistent with present knowledge to apply this formula generally.

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THE ROLE OF SALTS, HYDROGEN-ION CONCENTRATION AND AGAR IN THE RESPONSE OF THE AVENA COLEOPTILE TO AUXINS¹

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THE CONTINUED use of the *Avena* coleoptile in auxin studies has necessitated the closer examination of many points connected with it. The two procedures which have proved most useful are: (1) curvature produced by the one-sided application of auxin in agar to the decapitated coleoptile, (2) straight growth, produced either by the symmetrical application of auxin in agar to the decapitated coleoptile or by the immersion of isolated coleoptile sections in an auxin solution. The first detailed studies of these three methods were respectively those of Went (1928), Thimann and Bonner (1933), and Bonner (1933). The literature up to 1936 has been reviewed by Went and Thimann (1937). Additional data on the influence of the time interval between decapitation and application of the auxin-agar block have been furnished by Schneider and Went (1938), on the role of "physiological regeneration" by Skoog (1937), on the effect of light by van Overbeek (1936), and on the influence of sugar in straight growth by Schneider (1938).

The experiments reported here deal with (1) the technique of getting the auxin into the agar, and the influence of neutral salts thereon; (2) the role of pH—i.e., the relative activity of the auxin acid and its K or Na salt, both for curvature and for straight growth; (3) the effect of neutral salts on straight growth.

Since the sensitivity of *Avena* plants varies somewhat, it is essential, in experiments such as these, to compare only results obtained in the same test. For this reason, unless otherwise stated, all the data of any one table or figure were obtained at the same time.

In the curvature tests, the plants were grown in glass holders in the usual manner. Some of the important conditions have been varied in certain tests, particularly the interval of time between the decapitation and the application of the agar block (abbreviated to IDP). These variations are noted with the data. The plants were photographed 90 minutes after application of the agar blocks in all experiments, except those of table 7 in which the time was 110 minutes, and of figure 2B, in which it was 130 minutes. The tests were carried out in occasional red light at 24° and 85-90 per cent relative humidity.

For the soaking method, a 2.1 per cent agar gel was sliced 1.5 mm. thick and preserved in 70 per cent alcohol, being removed and washed in water as needed. All blocks were about 10 mm.³ in volume. In this method, auxin concentrations quoted refer to the solution in which the blocks were placed. In the pouring method, auxin concentrations refer to the final agar mixture.

RELATION BETWEEN AUXIN AND AGAR.—On the basis of an average activity for indole-3-acetic acid of 100,000 units per mg. (based on considerable numbers of determinations in the past), a concentration of 0.10 mg. per liter should give a curvature of about 10°. It was found, however, that if blocks of pure washed agar are soaked in solutions of pure indole-acetic acid dissolved in distilled water, the resulting angle was usually less than this. If tap-water was used as solvent, however, the curvature increased considerably. Table 1 gives averages of tests from several days. The effect is shown equally well with indole-acetic and indole-butyric acids.

Since the interval between decapitating and putting on the agar (IDP) has been shown by Schneider and Went (1938) to be of considerable importance in the *Avena* test, its influence in this connection was studied. Table 2 shows that the curvatures obtained

TABLE 1. *Avena* curvature produced by 2.1 per cent agar soaked in auxin solutions in distilled water or tap water. IDP, 3½ hours. Data collected from many separate experiments.

Auxin used	Concentration in mg./l.	Water for dilution		No. of plants per test
		Distilled	Tap	
Indole-3-acetic . .	0.1	7.0	13.5	48
Indole-3-butyric .	1.0	6.1	9.0	48
	1.0	7.0	10.1	72

with tap-water are about 9° higher than those with distilled water, irrespective of the length of this interval. In this and all other experiments (unless otherwise stated) a freshly made stock solution of indole-acetic acid, usually 100 mg. per liter, in distilled water, was diluted down to the test concentration with the water or salt solution to be studied.

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TABLE 2. *Avena* curvatures produced by 2.1 per cent agar soaked in auxin solutions made up in distilled water or in tap water.

	4 mg./l. auxin stock diluted to 0.08 mg./l. with	IDP in minutes		
		3	140	210
Distilled water		1.4	9.3	7.9
Tap water		10.7	16.9	18.3

The action of tap-water is doubtless due to its dissolved salts. The tap water in Cambridge contains 90-100 parts per million of dissolved salts. This effect of salts may be more conveniently studied by using a single pure salt; for this purpose KCl was chosen. Table 3 shows that if the auxin solution be made up with KCl (M/20 or M/100), blocks soaked in this mixture give very much larger curvatures than when soaked in pure auxin solution alone.

TABLE 3. *Avena* curvatures produced by 2.1 per cent agar soaked in auxin solutions made up with distilled water with and without KCl. IDP 40-80 minutes.

Indole-3-acetic acid concentration in mg./l.					
0.05			0.10		
Distilled water		KCl	Distilled water	KCl	
Oct. 4	4.0	8.0	Oct. 6	4.6	14.8
Oct. 4 ^a	1.7	8.8	Oct. 6 ^a	4.6	12.4
Oct. 13	0.0	9.0	Oct. 15	3.8	14.4
Oct. 18	2.6	12.8	Oct. 21	2.3	10.3
Dec. 1	1.1	9.2	Dec. 1	5.5	16.4
Average	1.9	9.6		4.2	13.7

^a Recrystallized sample.

A series of experiments with different concentrations of KCl indicate that the maximum effect is exerted at about M/100. Figure 1 shows the curvatures resulting from a constant concentration of indole-acetic acid in presence of varying concentrations of KCl and NaCl. It will be seen that KCl is more effective than NaCl, though both increase the curvatures markedly. It is also of interest to note that very low concentrations of salt, comparable to those in tap-water, exert a distinct effect. In another series of tests the chlorides of two other alkali metals were compared with KCl (table 4). The activity of LiCl is markedly low. By comparing these data with those of figure 1 and calculating to the same control value, it was deduced that the order of effectiveness is Li < Na < K > Rb.

The possibility that this effect might be due in some way to traces of toxic metals in the distilled water is ruled out by an experiment in which the action of KCl was compared in ordinary laboratory distilled water, in a sample of distilled water containing traces of copper (enough to inhibit the growth

TABLE 4. *Avena* curvatures produced by 2.1 per cent agar soaked in auxin solutions made up in different concentrations of Li, Rb, and K chlorides. Each value is an average from 12 plants except the control which is from 24. IDP, 40 minutes. Indole-3-acetic acid concentration, 0.08 mg./l.

Salt	Concentration of salt			
	M/100	M/1000	M/10,000	0
LiCl	4.5	3.2	1.3	
RbCl	5.8	5.3	1.3	1.8
KCl	9.2	—	—	

of aquatic fungi), and in freshly redistilled water from a Pyrex still (table 5). As table 5 shows, the increase of curvature by KCl is the same in each case, being of the order of 10^3 for these auxin concentrations.

TABLE 5. Effect of KCl on the soaking method with respect to three different samples of distilled water. Agar 2.1 per cent; IDP, 40 minutes.

Distilled water	Auxin concentration in mg./l.	Curvature		
		Without KCl	With KCl M/100	Increase
Laboratory supply	0.05	1.3	9.0	7.7
	0.10	9.4	19.8	10.4
Containing copper	0.05	0.8	7.7	6.9
	0.10	2.7	14.8	12.1
Redistilled in Pyrex	0.05	1.3	11.0	9.7
	0.10	4.3	18.8	14.5

Another observation bearing on the salt effect is the influence of the volume of solution used. The procedure of the preceding tables, which is that generally used in the soaking method, was to soak one or two blocks of agar, of a total volume less than 0.3 cc., in about 10 cc. of solution. It was found, however, that if only 0.5 cc. of solution was used, the resulting curvature was much increased, as shown

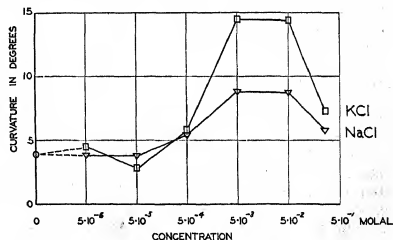


Fig. 1. Curvatures produced by blocks of 2.1 per cent agar soaked in 0.1 mg. per liter indole-acetic acid in the presence of varying concentrations of pure KCl and NaCl.

in table 6. For the small volume treatments listed here, the volume of solution was twice that of the added agar. The large volumes were 20 or more times as great.

TABLE 6. *Effect of the volume of solution in the soaking method. Agar, 2.1 per cent. IDP, 40 minutes. All curvatures, except the first, the mean of 24 plants. Each concentration tested on one day.*

Auxin concentration mg./l.	Large volume		Small volume	
	Without KCl	With KCl	Without KCl	With KCl
0.15	4.8	18.3	10.6	14.6
0.08	1.8	9.2	7.4	5.0
0.08	2.6	12.6	8.9	7.8

For large volumes, the results are in good agreement with the data of tables 5, 8, 9, and 10—i.e., the curvature is increased about 10° by the KCl. The results for small volumes, however, are rather unexpected. With both auxin concentrations, in the absence of KCl, the curvatures were increased by decreasing the volume of solution, as stated above—and this in spite of the diluting effect of the added agar. This increase in curvature must be ascribed to salts introduced with the agar; in the large volume these would be so diluted as to be relatively ineffective. It is, however, curious that the addition of KCl to the small volume gives no consistent effect for the two concentrations of auxin. It is perhaps possible that this is due to having reached the optimal salt concentration. In any event, the increase of curvature produced by the use of small volumes of salt-free solution is clear.

TABLE 7. *Comparison of pouring and soaking methods for three different IDP. Agar concentration 1.5 per cent.*

IDP in minutes	Auxin concentration in mg./l.	Curvature by method of	
		Soaking	Pouring
2	0.05	2.6	7.6
	0.10	10.4	17.5
40	0.05	5.5	10.0
	0.10	9.7	18.1
200	0.05	6.1	15.8
	0.10	14.4	18.8

The next step in the analysis of this salt effect was to compare the procedure of *soaking* agar in auxin solutions with that in which the agar is melted, mixed directly with the auxin solution, and *poured* into a mould. This method of direct mixing, which has been used by numerous workers as a standard procedure (cf. Nielsen, 1930; Dolk and Thimann, 1932) will be referred to as the "pouring" method. This method

gives curvatures about twice as great as those obtained in the soaking method, if everything else remains constant. Table 7 compares curvatures obtained by the two techniques at three values of the IDP. Although, in accordance with the findings of Schneider and Went (1938), the curvatures increase with increasing IDP, there is a 4° to 10° greater curvature produced by pouring than by soaking. The explanation of this increase will become apparent below.

It was found that with this "pouring" method the angle obtained is not further increased by the addition of KCl. Concentrations of KCl between M/200 and M/20,000,000 are completely without effect, while M/100, which is optimal for the soaking method, even slightly decreases the curvature (see, for instance, table 10). In table 8 the curvatures obtained by pouring are compared with those obtained by soaking agar in the auxin solution with or without KCl. It is clear that, for the concentrations of auxin and agar here used, the effect of KCl is to bring up the value obtained by soaking to about the level of that obtained by pouring.

Since KCl is without effect on the pouring method, it follows that its action must be exerted not upon the plant but upon the agar. For the same reason this action cannot be exerted upon the passage of auxin out of the agar into the plant, but must be an influence upon the uptake of auxin by agar.

An attempt to support this conclusion by analysis of the solution left after blocks had been soaked in it could not be carried out owing to the influence of small volumes of solution mentioned above (table 6). For if a large volume is used, then the change in auxin concentration caused by introduction of the agar blocks would be within the error of measurement, while with a small volume the effect of the KCl is largely nullified, as stated above.

However, the conclusion that the KCl acts upon the equilibrium between auxin solution and the agar is supported by an experiment of another kind, which demonstrates that such an equilibrium does exist. The experiment consisted in allowing agar which contained a high auxin concentration at the start to come into equilibrium with a lower concentration in the surrounding solution. Blocks of 1.5 per cent agar, containing 0.1 mg. indole-acetic acid per liter (by pouring) were soaked for one hour in two lower indole-acetic acid concentrations—namely, 0.025 and 0.050 mg. per liter. For comparison, plain 1.5 per cent agar blocks were soaked in samples of the same solutions. Table 9 shows that agar previously enriched in auxin gave no higher curvatures than those not enriched. Thus the same equilibrium value is reached whether the blocks give auxin out to the solution or take it in from the solution. This equilibrium is evidently reached within one hour, which is the standard time used in all soaking experiments.

It is clear, then, (for the concentration ranges that we have studied) that at the point of equilibrium the auxin is unequally distributed between the agar and

the solution. Salts bring the point of equilibrium more nearly to that of equal distribution, KCl being most effective of those tried. The explanation for this effect doubtless lies in some colloidal properties of the agar. This has led us to reconsider the importance of the concentration of the agar.

EFFECT OF AGAR CONCENTRATION.—As might be anticipated from the above discussion, the concentration of agar plays an important role in the *Avena* test. Insufficient attention appears to have been paid to

auxin concentration of 0.1 mg. per liter gave, in 1 per cent agar, 14.9°, and in 6 per cent agar only 1.6°. Correspondingly in presence of KCl M/100, the values were, in 1 per cent agar, 21.3°; in 6 per cent agar, 8.9°.

A more complete comparison is given in table 10 in which the influence of agar concentration, both with and without KCl, is shown for the soaking and pouring techniques. The data show that a concentration giving a curvature as high as 11° in 1 per

TABLE 8. Comparison of soaking and pouring methods with and without KCl M/100. IDP 35-50 minutes.

Date (1937)	Indole-3- acetic acid concentration in mg./l.	Agar concentration in %	Soaking method		Pouring method	
			Without KCl	With KCl	Without KCl	With KCl
Oct. 27	0.05	2.1	0.8	5.8	5.4	5.3
Oct. 27	0.10	2.1	4.9	11.1	14.8	9.8
Dec. 11	0.05	1.5	—	16.3	13.3	—
Dec. 17	0.05	1.5	8.0	18.6	18.8	—

TABLE 9. Effect of previous enrichment of agar with indole-3-acetic acid on its subsequent attainment of equilibrium with aqueous solutions. Agar, 1.5 per cent. IDP, 60 minutes.

Auxin content of agar block before soaking mg./l.	Curvatures after soaking one hour in auxin solution			
	Auxin 0.025 mg./l.		Auxin 0.050 mg./l.	
	Without KCl	With KCl	Without KCl	With KCl
0	1.6	4.5	5.5	8.5
0.1	1.4	5.3	3.9	7.4

this factor in the past. Du Buy (1931) mentions that very high agar concentrations reduce the curvature. The data of table 8 show that 1.5 per cent agar gives larger curvatures than 2.1 per cent.

In a preliminary experiment extreme agar concentrations were compared by the soaking method. An

TABLE 10. Effect of agar concentration, with and without KCl, M/100, for pouring and soaking methods with two indole-3-acetic acid concentrations. IDP, 35 minutes.

Method	Auxin concentration in mg./l.	Curvature					
		Without KCl			With KCl		
		Percent agar	Percent agar	Percent agar	Percent agar	Percent agar	Percent agar
Soaking	0.05	5.9	0.8	+0.3	12.3	5.8	2.6
Soaking	0.10	11.1	4.9	0.7	16.3	11.1	9.5
Pouring	0.05	9.7	5.4	4.2	9.2	5.3	1.9
Pouring	0.10	15.3	14.8	10.0	13.8	9.8	6.5

cent agar may become scarcely detectable when 4 per cent agar is used. The differences are most marked in the soaking technique, but are very great

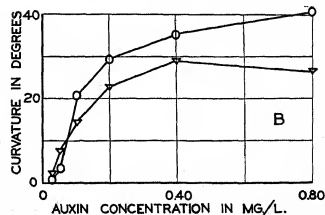
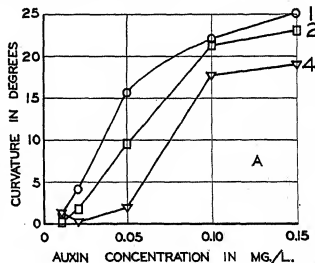


Fig. 2. The relation between *Avena* curvature and indole-acetic acid concentration.—A (above). Experiment of Dec. 15, 1937, using 1, 2, and 4 per cent agar. Pouring method. IDP, 40 minutes. Photographed at 90 minutes.—B (below). The same but with IDP 2 minutes. ▽, 1.5% agar, Dec. 27, 1937; ○, 2% agar, Feb. 8, 1938. Photographed at 130 minutes.

in all cases. By decreasing the agar from 2 per cent to 1 per cent, the curvature obtained is approximately doubled. Considering that 1 and 2 per cent agar are 99 and 98 per cent water, respectively, so great a difference is rather unexpected. It is also important that the ratio between the angles obtained with 0.05 and 0.10 mg. auxin per liter is by no means constant — i.e., the proportionality curve between curvature and auxin concentration is strongly affected by the concentration of agar used.

The data of figure 2A illustrate this last point more clearly. Here a series of auxin concentrations in 1, 2, and 4 per cent agar was studied by the pouring technique. It is evident that the effect is not merely the shifting of the proportionality curve towards the right with increasing agar concentration, but that it also involves a change in the whole relationship between auxin concentration and curvature. Thus 1 per cent agar gives a markedly rounded curve, 2 per cent shows a nearly linear proportionality, while 4 per cent gives a sigmoid curve with a considerable "threshold." Another experiment, giving similar results, showed further that the magnitude of the maxi-

mum angle is not decreased by increasing the agar concentration to 4 per cent, but that the auxin concentration necessary to reach it is greatly increased. Thus in 1 per cent agar the maximum angle of 23° was produced by 0.15 mg. indole-acetic acid per liter, while in 4 per cent agar the same maximum angle was attained at about 0.32 mg. per liter. The finding that the relation between curvature and auxin con-

centration may, under some conditions, be a smooth curve is further supported by data obtained with a very short IDP. Here the tendency to a smooth curve is greater than with IDP of 40 minutes or more, so that even 2 per cent agar gives a rounded curve. Figure 2B summarizes data obtained on two occasions, with 1.5 and 2 per cent agar, respectively. Both curves are rounded.

On the whole, the effect of agar concentration is at least as great as that of the IDP; it is also at least as great as the variation in sensitivity of the *Avena* test from day to day.

INFLUENCE OF HYDROGEN-ION CONCENTRATION.—The experiments recorded in the literature are consistent in indicating that in the absence of buffer, pH exerts no influence on the response to auxin. Thus Nielsen (1930) found the curvature approximately the same between pH 2.4 and 9.6. Thimann (1935) found that the sodium salts of several auxins were fully active in promoting straight growth in immersed sections. Jost and Reiss (1936), also studying straight growth, found no change in activity on acidifying indole-acetic acid solutions. On the other hand, auxin solutions buffered at pH greater than 7 give much smaller curvatures than at pH 5 (Dolk and Thimann, 1932) (see also Bonner, 1934). By contrast, the induction of root formation by auxin shows no effect of pH even with buffering (Went, 1934).

It has recently been reported by Avery, Burkholder, and Creighton (1937a) that the potassium salts of indole-acetic, indole-butyric, and naphthalene-acetic acids are more active than the free acids. The effect was of the order of 2 to 10 times and was obtained in unbuffered solutions. Their data, however, are somewhat inconsistent in that they also mention that neutralizing the solution of the acid to pH 7 did not affect its activity. Bringing to pH 7 would, of course, convert these acids to their salts. The increased activity of the salts was partially confirmed in the same laboratory by Scheer (1937) for straight growth measurements. Because of the disagreement between these results and the facts cited above, it was thought worth while to reinvestigate the relative activities of auxin acids and their salts.

It is clear, especially in view of the effects of neutral salts disclosed in our experiments, that comparison of the activity of a sample of acid with a sample of its salt is not valid unless both are equally pure. In our experiments, therefore, we have used only the pure acid (twice recrystallized) and compared the activity of its solutions with that of the same solution neutralized with the calculated quantity of standard KOH to pH 7.

The results of 28 tests, each with 24 plants, are collected in table 11. All these values were obtained by the soaking technique in the absence of KCl and with long IDP. Other tests with shorter IDP also showed no difference between the activity of the acid and its salt. Since the technique used by Avery, Burkholder, and Creighton was that of pouring, similar experiments were carried out by pouring, using

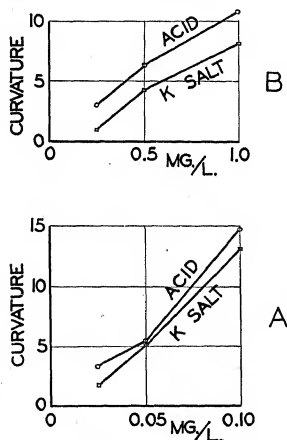


Fig. 3. *Avena* curvatures produced by free auxin acids and their potassium salts; A, indole-acetic, B, indole-butyric. Pouring method. Agar 1.5 per cent. IDP, 40 minutes.

um angle is not decreased by increasing the agar concentration to 4 per cent, but that the auxin concentration necessary to reach it is greatly increased. Thus in 1 per cent agar the maximum angle of 23° was produced by 0.15 mg. indole-acetic acid per liter, while in 4 per cent agar the same maximum angle was attained at about 0.32 mg. per liter. The finding that the relation between curvature and auxin con-

TABLE 11. *Avena* curvatures produced by 2.1 per cent agar soaked in neutralized and not neutralized auxin solutions in distilled water and in tap water. IDP about 3½ hours.

Water for Dilutions	Concentration in mg./l.	Not neutralized	Neutralized	Difference	No. of tests
Indole-3-acetic acid					
Distilled	0.042	3.3	3.6	+0.3	2
	0.050	3.4	3.7	+0.3	2
	0.083	7.8	7.9	+0.1	2
	0.10	6.9	7.6	+0.7	6
	0.33	19.7	17.7	-2.0	2
Tap	0.10	9.3	10.1	+0.8	3
Indole-3-butyric acid					
Distilled	0.30	5.1	4.0	-1.1	2
	1.0	8.1	7.0	-1.1	4
	2.5	12.6	10.0	-2.6	2
Tap	1.0	9.3	10.1	+0.8	3

1.5 per cent agar, which, as shown above, is the most favorable concentration. These results are summarized in figure 3, A and B.

Thus, neither for indole-acetic nor for indole-butyric acid is there any considerable difference between the activity of the pure auxin acid and its salt. If anything, there is a tendency for the salt to be somewhat less active than the acid. Similar results have just been published for indole-acetic and its Na and K salts by D. M. Bonner (1937).

The same relationship was investigated for the straight growth of immersed sections. In this test growth is approximately proportional to the logarithm of the auxin concentration over a considerable range. However, indole-acetic acid concentrations of about 50 mg. per liter cause a reduction in growth, and still higher concentrations bring about an actual shrinkage. These effects are evidently due to some kind of toxicity of these high concentrations (cf. fig. 5 in Thimann and Sweeney, 1937). The sole effect of salt formation is in the reduction of this toxicity. Figure 4 shows a representative experiment. Other experiments gave similar results. It may be seen that at concentrations up to 10 mg. per liter (the physiological range) the salt is no more effective than the acid. At 40 mg. per liter or higher the salt is less toxic than the acid, but at 800 mg. per liter the salt is also toxic.

A further comparison between the activity of the auxin acid and its salt was made possible by the experiments on protoplasmic streaming (Thimann and Sweeney, 1937). Using the technique described in that paper, the effect of indole-acetic acid, neutralized and unneutralized, was compared for several concentrations.² Figure 5 shows the "total effect"—i.e., the increase or decrease in streaming rates over those of the controls. It will be seen that there is here a

² Experiments kindly carried out by Mrs. B. M. Sweeney.

very marked difference; the curve for the salt is shifted over to the right of that for the acid. Thus the range of concentration in which the salt causes acceleration of streaming is about 100 times that in which the acid has the same effect. The effect of the salt is thus in the opposite direction to the effect in the curvature test claimed by Avery, Burkholder, and Creighton and discussed above—namely, the salt is here less active than the acid.

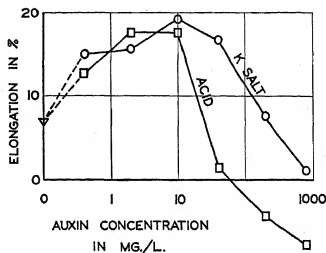


Fig. 4. Percentage elongation of 3 mm. sections of *Avena* coleoptiles in various concentrations of indole-acetic acid and its potassium salt. Measured at 36 hours. Each point is the mean of 40 sections (10 plants), except the water control, which is from 80.

The following is a possible explanation of this considerable reduction in activity: In streaming experiments the effect is observed and measured immediately after application of the auxin. Since fructose was not present, the whole effect was over within about 30 minutes. The visible effect on streaming is therefore very dependent upon rapid entry of the active substance into the cell, and an auxin which enters only slowly would cause the acceleration of streaming to be spread over so long a time that it could not readily be detected. Thus, although the final growth

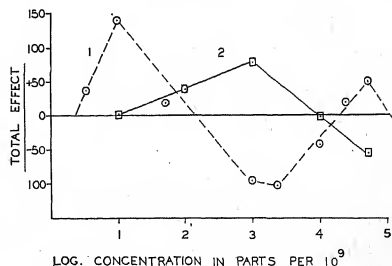


Fig. 5. Effect on protoplasmic streaming of epidermal cells of the *Avena* coleoptile of indole-acetic acid (curve 1) and its potassium salt (curve 2). Each point is derived from a complete curve of streaming rate against time after auxin application.

response, measured over a longer time, would be the same (or, if anything, slightly less for the salt than for the acid), the immediate streaming response is not detectable. Since the salt is ionized, it would be expected to enter more slowly than the unionized free acid. The pK of the acid is at 4.8, so that at pH 7 the concentration of free acid is about 1/100 of that of the salt. Correspondingly, as pointed out above, the salt behaves as though its concentration were reduced 100 times.

INFLUENCE OF NEUTRAL SALTS ON STRAIGHT GROWTH.—In the first section of this paper it was shown that the presence of KCl may greatly increase the curvature obtained in the *Avena* test, but that this results from its effect on the uptake of the auxin by the agar. During the 2 hours in which the block containing auxin and KCl was in contact with the coleoptile, no growth promoting effect of the KCl itself could be observed. However, since this was a very short period, experiments were conducted over longer times to determine whether KCl actually plays any part in growth or not. For this purpose the straight growth of sections, as first described by Bonner (1933) but with the technique described by Schneider (1938), was used.

Sections were cut 3 mm. long by means of a cutting machine, the zones 6, 9, and 12 mm. below the tip being used. They were mounted on combs and each comb immersed in 40 cc. of solution in a Petri dish. Lengths were measured under a microscope with ocular scale, using a total magnification of $16\times$.

Figure 6 shows the elongation of sections, in percentage of their initial length, in a series of KCl concentrations, each containing 1 mg. per liter indole-acetic acid and 1 per cent sucrose. The effect of the higher concentrations is noticeable at least as early as 15 hours, and at longer times it may be seen that even $M/10,000$ KCl definitely increases the growth

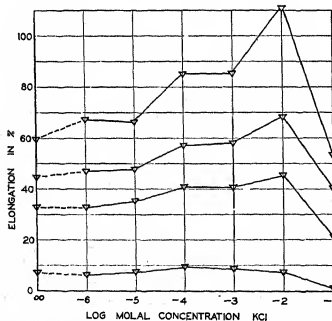


Fig. 6. Percentage elongation of 3 mm. sections of *Avena* coleoptiles in 1 mg. per liter indole-acetic acid and 1 per cent sucrose plus serial concentrations of KCl . Measured at 2, 15, 24, and 63 hours after cutting.

over that in controls. The optimum KCl concentration, $M/100$, almost doubles the growth. In this same experiment $M/100$ KCl also considerably increased the growth in the absence of sugar. Thus at 63 hours the controls in auxin alone (in distilled water) grew 20 per cent; those in auxin in tap water, 38 per cent, and those in auxin in $M/100$ KCl , 73 per cent.

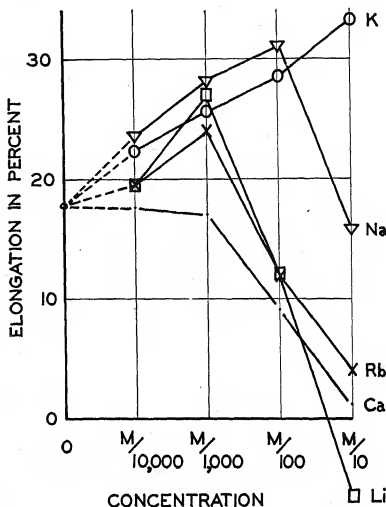


Fig. 7. Percentage elongation of 3 mm. sections of *Avena* coleoptiles in 1 mg. per liter indole-acetic acid plus serial concentrations of chlorides of calcium and the alkali metals (no sugar added). Each point is the mean of 30 sections (10 plants) except the control which is from 60.

In figure 7 are compared the activities of Ca and of the alkali metals Li , Na , K , and Rb , all as chlorides in the presence of 1 mg. per liter indole-acetic acid. The effects of all the salts are somewhat less than in figure 6, but the conclusions are clear. At the concentration $M/10$, $LiCl$, $RbCl$, and $CaCl_2$ were markedly depressive, while $NaCl$ was less so, and KCl gave excellent growth. At $M/100$, $NaCl$ was about as effective as KCl or slightly more so. At $M/1000$, $LiCl$ and $RbCl$ show weak effects. Taking the data as a whole, the order of relative effectiveness of these chlorides is: $Ca < Li < Rb < Na < K$. In the same experiment $Ca(NO_3)_2$, $M/100$, was also slightly depressive, so that the action cannot be regarded as one of nutrition. It may be noted that with this technique, rubidium chloride has no particular activity in promoting growth (cf. Avery, Burkholder,

and Creighton, 1937b). The depressive effect of CaCl_2 is paralleled by the recent experiments of Borris (1937) on the growth of *Spergula* seedlings.

To determine whether KCl exerts this effect under all conditions, further comparisons were made. Auxin, sucrose, and KCl each has a range of somewhat over 100-fold over which growth in the section test is a function of their concentrations. For each substance, therefore, a low concentration giving a just detectable effect and one 100 times as high, giving the optimal effect, were selected. In a long series of tests, all carried out at the same time, these two concentrations of auxin, sucrose, and KCl were combined in

gradient is really the controlling factor. It is often tacitly assumed that the force causing cell enlargement is an osmotic force, and the use of the section test makes a demonstration of this easy. If, instead of KCl, we use a highly impermeable substance which is not also a food factor, then its presence in the external solution will not result in an increase of the internal osmotic pressure, but will merely reduce the osmotic gradient. Thus, any increase of the external concentration of this substance must, by reducing the osmotic gradient, reduce growth.

For this purpose mannitol was selected. Its rate of entry into the cell sap is extremely slow (Collan-

TABLE 12. Comparison of auxin, sugar, and KCl separately and together on the section test. Percentage growth in 36 hours. Each value is the mean of 30 sections (10 plants) except those marked * which are means of 60.

Auxin in mg./l.	0	0	0	0.01	0.01	0.01	1.0	1.0	1.0
Sugar in per cent	0	0.01	1	0	0.01	1	0	0.01	1
Distilled water	11*	8	18	11	11	30	19*	31	52*
KCl M/10,000	9	14	19	17	18	34	22	25	58
KCl M/100	10*	9	15	14	14	30	33*	44	56*

all possible ways. The number of sections was large enough to make small differences significant. The results are collected in table 12, expressed in percentage of initial length of the sections. The following conclusions may be drawn: (1) Addition of KCl does not improve the growth of controls without auxin. This held for several other experiments, but one notable exception, in which the growth of controls was definitely increased by M/100 KCl, suggests some variation in the response. (2) In the presence of sugar alone the action of KCl is less than in the presence of auxin alone. The high sugar concentration, alone or with low auxin, prevents or reduces the action of KCl. (3) In high auxin concentration the high concentration of KCl is more effective; in low auxin concentration the reverse is true. This holds both in the presence and absence of sugar.

There is considerable variation in the effectiveness of KCl from experiment to experiment, so that the above generalizations must be regarded as tentative.

These facts suggest that the effect of KCl on coleoptile growth is as a contributor to osmotic pressure. But since sugar alone increases growth (in the presence of auxin) more than does KCl alone, and since also KCl increases the growth even in the presence of optimal sugar and optimal auxin (see fig. 6), it is clear that the effects of KCl and of sugar are different. The findings are completely consistent with the view that the salt meets the osmotic requirement, while the sugar, which may also partly act osmotically, is in the main a food factor. The readiness with which potassium is known to enter the cell is also in line with this view. Another possible interpretation is mentioned in the discussion.

In connection with the physiology of growth it is important, however, to know whether the osmotic

der and Bärlund, 1933), and it is also not a food factor (Schneider, 1938). Figure 8 shows the growth, after 24 and 72 hours, of sections in optimal auxin and KCl, in the presence of a series of concentrations of mannitol. The straight line relationship shows that the growth is directly proportional to the osmotic gradient. Further, the effect is not due to a toxicity because all sections grow as much as the controls during the period between 24 and 72 hours. Even the shrunken sections recover somewhat during

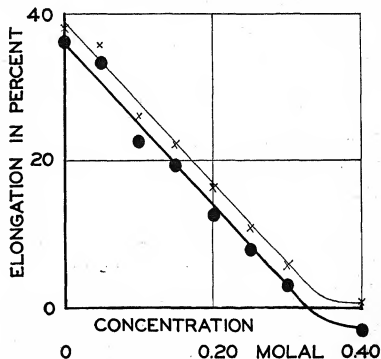


Fig. 8. Percentage elongation of 3 mm. sections of *Avena* coleoptiles in 1 mg. per liter indole-acetic acid and M/100 KCl plus increasing concentrations of mannitol. Each point is the mean of 30 sections (10 plants), except the control, which is the mean of 60. Heavy line curve, 37 hour reading; fine line curve, 73 hour reading.

this second period. This provides, we believe for the first time,³ a simple demonstration that irreversible cell enlargement—that is, normal growth—is directly proportional to the osmotic gradient.

A final point of interest in connection with the test remains to be mentioned. Sections cut from plants growing in the dark elongate considerably more than those from plants growing in red light and still more than do sections from plants growing in white light. Table 13 shows this effect both in controls and in the presence of auxin, KCl, and sugar. The plants were given red light for the first 30 hours to suppress the mesocotyl growth and thereafter placed in darkness, red light (60 watt ruby bulb at 1.5 meters), or white light (400 watts at 0.8 meters). Later, sections were cut (at a total age of 74 hours), and the subsequent growth of these in the solutions was in all cases in darkness with occasional red light. The very large growth of dark-grown sections in auxin-KCl-sugar is especially striking.

TABLE 13. Percentage elongation of sections cut from plants grown under different light conditions. Measured at 94 hours.

Percentage elongation of sections when placed in				
Plants grown in	Water	Auxin 1 mg./l.	Auxin 1 mg./l. + KCl M/100	Auxin 1 mg./l. + KCl M/100 + sucrose 1%
Darkness	26	59	67	133
Red light	21	51	61	83
White light	13	27	43	73

While the sections were in the solutions, a few plants were left intact to continue growth, and when their coleoptiles had reached their final lengths, these were measured. Left to grow in the dark, the coleoptiles reached a final length of 5.1 cm., those in red light, 4.7 cm., and those in white light, 3.6 cm. Hence the amount of elongation which the sections undergo after cutting is roughly proportional to the amount of elongation which they would have undergone had they remained on the plant.

The explanation for this effect is by no means clear. It cannot be due to photodynamic inactivation of auxin, since the sections are subsequently kept in optimal auxin concentration. For the same reason it can hardly be due to deficiency of sugar. It may be connected with some highly light-sensitive reaction.

In any event, the fact is of empirical importance, since in order to obtain maximal growth of isolated sections, they should be cut from plants first treated with red light and then grown in darkness.

DISCUSSION.—The experiments in section 1 have shown that the equilibrium between auxin and agar is not one of simple distribution, but that the auxin concentration is higher in the aqueous phase than in

the agar. In presence of some neutral salts (KCl being better than NaCl, LiCl, or RbCl) the auxin concentration in the agar can be raised to the point of equal distribution; this is shown by the identity between the results obtained by soaking agar in auxin with KCl and by direct mixing of auxin solution with melted agar. An important empirical consequence of this is that the soaking method is not valid for comparisons between pure salt-free auxins and impure mixtures. It is also not valid for comparisons of different pure auxins, since there is no certainty that the distribution of different substances between water and agar would be the same. In fact, there seems little justification for the continued use of the soaking method at all.

These findings explain the difficulty encountered by Kögl, Haagen Smit, and Erxleben (1933), who found that after auxin was partially purified its apparent activity fell off sharply but was restored by the addition of 0.002 M KCl and acetic acid. So long as the

auxin was very impure, the addition of the salt was not necessary. These workers, of course, used the soaking method. Similar anomalous results may be seen in the data of D. M. Bonner (1937) who records curvatures in the presence of phosphate buffer 3-14° higher than in the absence of salts.

The properties of the agar seem to have been largely overlooked in regard to the *Avena* test. Not only is its effect on auxin distribution of major importance, but the influence of the agar concentration is also very large. The shape of curve obtained when curvature is plotted against auxin concentration is largely dependent on the concentration of the agar. A typical Blackman curve, with two straight arms and a very short transition zone, is given (for the 40-minute IDP) by 1.5 per cent agar in the pouring technique. This is, therefore, about the most suitable concentration for the pouring method and, as it happens, was used by Nielsen (1930) and Dolik and Thimann (1932). For the soaking method, at the 40-minute IDP, the same can be attained with 2 per cent agar by addition of KCl, M/100. At low agar concentrations, especially with very short IDP, the curve is, on the other hand, approximately logarithmic. It is noteworthy that for the various methods of auxin-agar preparations studied, the effect of increasing IDP persisted (see table 2, 7)—i.e., the effect of IDP is due to changes in the plant itself, while

³ Dr. J. van Overbeek (private communication) has obtained similar results with *Zea mays* coleoptiles, using sucrose.

effects of neutral salts and agar concentration must be concerned instead with the colloidal properties of the agar.

It is of interest to note that the slightly sigmoid curve shown by Söding (1936) for the (daylight) *Avena* test resembles that given by 4 per cent agar in the pouring method (fig. 2A) which is even more sigmoid; Söding states that 3 per cent agar was used but mentions a tendency to shrink on keeping. On the other hand, the rounded curves of figure 2B resemble the curve of Nielsen (1930). Nielsen used 1.5 per cent agar and zero IDP, which are precisely the conditions of one of the curves of this figure. Possibly many of the differences described in the literature are due to use of different agar concentrations. By suitably varying both agar concentration and the IDP, it is possible to obtain curves of very different shapes.

It is perhaps scarcely necessary to add, therefore, that little theoretical significance can be placed on the shape of the curve as an indicator of a Blackman limiting-factor system in the *Avena* test. The smoothly rounded curve of 1 per cent agar or the sigmoid curve of 4 per cent (fig. 2A) might equally well be given significance and different deductions drawn.

In regard to the effects of pH, our results agree with those of D. M. Bonner (1937) and those found in earlier experiments—namely, that in unbuffered solutions, bringing the auxin to pH 7 has no effect on the activity in the *Avena* test. The contrary findings of Avery, Burkholder, and Creighton (1937a) are presumably to be attributed to different degrees of purity of the products used. No criteria of chemical purity are given by them for any substances.

Certain differences in activity between the auxin acid and its salt do exist, however. Firstly, the salt is less toxic at very high concentrations in straight growth measurements, as was also reported by Scheer (1937). But in her experiments the potassium salt of indole-acetic acid was apparently more active at all concentrations than the free acid. On the other hand, the growth of her plain agar controls used for the physiological concentrations of indole-acetic acid was nearly 2 mm. greater than that of the similar controls for the potassium salt; this difference in the controls could account for the bulk of the apparent difference in activity. In the case of naphthalene-acetic acid, the controls were very nearly the same for the acid and the salt, and correspondingly, there was found to be little systematic difference between the activity of the acid and that of the salt, except at the highest concentrations, in which the salt showed less toxicity.

Secondly, as is shown by the data on protoplasmic streaming, the auxin salt apparently enters the cell less rapidly than the acid. This effect is in line with the general findings on permeability, according to which ionizable substances penetrate more readily in the unionized than in the ionized form. Since the auxins are weak acids, their salts will be more completely ionized than the free acids.

The finding that KCl and, to a lesser extent, other salts, have an important influence on the growth of isolated coleoptile sections is of considerable interest. To the substances already known to control growth in the coleoptile—namely, auxins and sugars—neutral salts such as KCl must now be added. It is probable, though not certain, that the effect of salts in this way is an osmotic one, the salt serving to maintain the osmotic pressure in tissues which, because of their continued enlargement in salt-free auxin solution, would otherwise become poor in osmotic material. Another explanation, however, is possible. The numerous recorded effects of neutral salts on colloidal and enzymatic systems suggest that the action of KCl and other salts on growth might be of the nature of such "salt effects"—i.e., through their influence on protein hydration, permeability, etc. The resemblance of the series Ca, Li, Rb, Na, K to the Hofmeister series certainly supports this possibility. Another remarkable fact is that the effectiveness of the salts in promoting equal distribution of auxin between water and agar probably follows a similar order. For the present, therefore, the mechanism of this salt action must be left open. In future testing of substances for their activity as auxins by the section test, however, the addition of optimal concentrations of KCl and sucrose is an obvious recommendation.

SUMMARY

When blocks of plain agar are soaked in simple solutions of indole-3-acetic acid or indole-3-butyric acid, the concentration of auxin in the agar does not reach that in the aqueous phase. Addition of KCl, or, to a lesser extent, of other salts, or even of tap water, increases the concentration of auxin in the agar. The optimal KCl concentration, M/100, increases the auxin concentration in the agar up to the level of that obtained by direct mixing of the solution with melted agar.

The sensitivity of the *Avena* curvature test is greatly increased by lowering the concentration of the agar used.

The type of relationship between auxin concentration and curvature is also dependent on the concentration of agar used, and it is deduced that the straight line relationship which can be obtained under certain conditions is of empirical rather than theoretical significance.

In the *Avena* curvature test the activity of indole-acetic and indole-butyric acids is not lower than that of their potassium salts.

In the straight growth of immersed coleoptile sections, indole-acetic and indole-butyric acids are more toxic at extremely high concentrations than their salts, and there is evidence that this is associated with their more rapid rate of entry into the cell.

In the straight growth of immersed coleoptile sections, neutral salts are an important factor, especially in the presence of auxin. Their action is not nutritive but is probably due either to their entry and subsequent contribution to the osmotic pressure of the tissues or to their action upon the colloid prop-

erties of the tissues. In the chlorides the order of effectiveness is $\text{Ca} < \text{Li} < \text{Rb} < \text{Na} < \text{K}$. This effect is to be clearly distinguished from the effect of neutral salts on the auxin-agar relationship.

The growth of immersed coleoptile sections is directly proportional to the osmotic gradient between their contents and the external solution.

Sections cut from coleoptiles grown in the dark elongate more in auxin solutions than those from coleoptiles grown in red or white light.

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THE EFFECT OF COLCHICINE ON MICROSPORE MOTHER CELLS AND MICROSPORES OF TRADESCANTIA PALUDOSA¹

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THE VARIOUS factors which have induced chromosome doubling in plants have been summarized by Sax (1937). Probably the earliest work on the use

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This study was made while the writer held a fellowship at Radcliffe College from the American Association for University Women. She is indebted to Professor Karl Sax for suggestions and criticisms during the preparation of this paper.

Since this manuscript was prepared, B. R. Nebel and M. L. Ruttie have published "Cytological and genetical significance of colchicine" in the *Journal of Heredity*, 29: 3-9, 1938.

of chemicals in the production of polyploid cells is that of Némec (1904), who treated roots of pea, bean, and onion with chloral hydrate. Similarly, tetraploid cells have been obtained by use of chloral hydrate in *Hyacinthus candicans*, *Vicia faba*, and *Pisum sativum* (Kemp, 1910), in *Vicia faba*, *Pisum sativum*, and *Zea mays* (Sakamura, 1920), in *Vicia faba* (van Regemorter, 1926), and in *Pisum sativum* (Peto, 1935). Polyploid pollen grains have been observed in *Lycium halimifolium* and *Datura ferox* as a result of attack of flower buds by insects (Kostoff and Kendall, 1929, 1930) and in *Nicotiana tabacum* and

N. triplex as a result of infection by the tobacco mosaic virus (Kostoff, 1933).

The effects of colchicine on animal tissue are many and have been known for several years. Dustin (1934) and Lits (1934, 1936) showed that injections of colchicine in mice stimulate mitoses in all tissues where divisions normally occur. This is followed by a period of nuclear inactivity. Dustin, Havas, and Lits (1937) found that when roots and plumule of wheat and roots of shallot and tulip are treated with low concentrations of colchicine, growth is accelerated, whereas with stronger concentrations of colchicine, growth is retarded. Microscopic studies showed that the chromosomes are distinct and separated at metaphase, but fail to pass to the poles. A single large polylobed nucleus is formed. Havas (1937) observed that when certain concentrations of colchicine are used with dialysate viscum on wheat seedlings, a definite increase and subsequent marked decrease of growth occurs in roots and root hairs.

Brues (1936) and Brues and Cohen (1936) found that mitoses are inhibited in the regenerating liver of rat after suitable treatment with colchicine, octahydrocolchicine, *n*-acetylcolchinal, and four derivatives of the latter. The number of mitoses seen in a retarded condition is equal to the number of mitoses which normally would have occurred during that time. The onset of division is inhibited. Nebel (1937) found that colchicine inhibits mitosis in the stamen hairs of *Tradescantia* and in the developing egg of *Arabia punctulata*. Tetraploid cells are produced in *Tradescantia* stamen hairs from the reconstruction of a single nucleus of a blocked metaphase. Phenyl-, amyl-, propyl-, and ethyl-urethane and chloral hydrate produce binucleate cells occasionally. Cuttings and seedlings of *Zea*, *Vicia*, *tomato*, *Tagetes*, *Antirrhinum*, *Trifolium*, *Papaver*, *Dianthus*, *Solanum*, and *Lilium* showed marked reactions to colchicine (Ruttle). Tissues resulting from treated meristems showed irregular growth, incised and crumpled leaves, and chlorophyll defects. Cytological studies showed multinucleate cells.

Blakeslee (1937) obtained diploid and tetraploid pollen grains from plants of *Datura stramonium*, *Portulaca*, and *Cucurbita* which were treated with colchicine. By various methods of treatment he has induced changes in more than twenty other species of plants which he has interpreted to be due to chromosome doubling. These plants have thickened stems and broad, roughened leaves.

MATERIALS AND METHODS.—Cuttings of flowering stalks of *Tradescantia paludosa* Anderson and Woodson were placed in 0.2, 0.1, 0.05, 0.025, and 0.01 per cent aqueous solutions of colchicine (Merck) for twenty-four hours and in 0.05 per cent solutions for forty-eight hours. About three cubic centimeters of solution were allowed for each flower stalk. The treated stalks were transferred to tap water and placed in the greenhouse. Untreated flower stalks were placed in tap water for controls. Daily observations were made of the microspore mother cells and

the microspores. Preparations were made by Belting's aceto-carmin technique as modified by Zirkle (1937) and by a modification of Taylor's smear method. In the latter method Flemming's medium and Karpechenko's modification of Navaschin's solutions were used as fixatives. The material was stained by a modification of Newton's crystal-violet-iodine method. Flower buds were also fixed in Navaschin's and Flemming's medium solutions. The fixed material was imbedded in paraffin. Sections were cut ten microns in thickness and stained with Flemming's triple stain.

All photographs were made at a magnification of 1200 \times . Drawings were made with a camera lucida at table level. Bausch and Lomb 12.5 \times compensating ocular and Bausch and Lomb 1.8 mm. 1.30 N.A. fluorite oil immersion objective were used.

OBSERVATIONS.—The normal diploid chromosome number of this strain of *Tradescantia paludosa* is six pairs of normal chromosomes and one pair of fragments. Meiosis and pollen grain development in this species (pl. 1, fig. 1) are similar to those described by Sax and Edmonds (1933) for other species of *Tradescantia*. The meiotic divisions are regular, and the pollen fertility is high.

Examination of material treated with 0.025 and 0.01 per cent solutions of colchicine show no evident effect on the microspore mother cells and microspore, while concentrations of 0.05, 0.1, and 0.2 per cent inhibit meiosis temporarily. The length of time required for recovery from the treatment appears to increase with the concentration of colchicine used. Concentrations of 0.05 and .1 per cent gave most uniform results. All descriptions are taken from material treated in this way.

Examination of microspore mother cells twenty-four hours after treatment shows that the six pairs of chromosomes and one pair of fragments which are paired normally at the first metaphase fail to pass to their respective poles, but form a contracted mass at the equatorial plate (pl. 1, fig. 2). The cytoplasm is uniformly granular, and spindle fibers are not visible at this stage. This contracted condition apparently persists for several days. The chromosomes of the microspore mother cells undergo a resting period, during which there is a gradual increase in size of the cell, an elongation of the chromosomes, and an uncoiling of the major spirals (pl. 1, fig. 3, 4). Cells containing twelve univalents are formed. In the majority of cases, these divide equatorially (pl. 1, fig. 5) forming the twelve chromosomes which are found in each dyad. The latter develop independently to form diploid pollen grains (pl. 1, fig. 9). In rare instances pollen grains were formed in which there were eleven or thirteen chromosomes, presumably caused by non-disjunction at the equatorial division.

Observations of material four days after treatment show instances in which the first meiotic division has occurred, but cell division is apparently inhibited, and the cell wall is not formed (fig. 1). In some instances cytokinesis is initiated, but later arrested (fig.

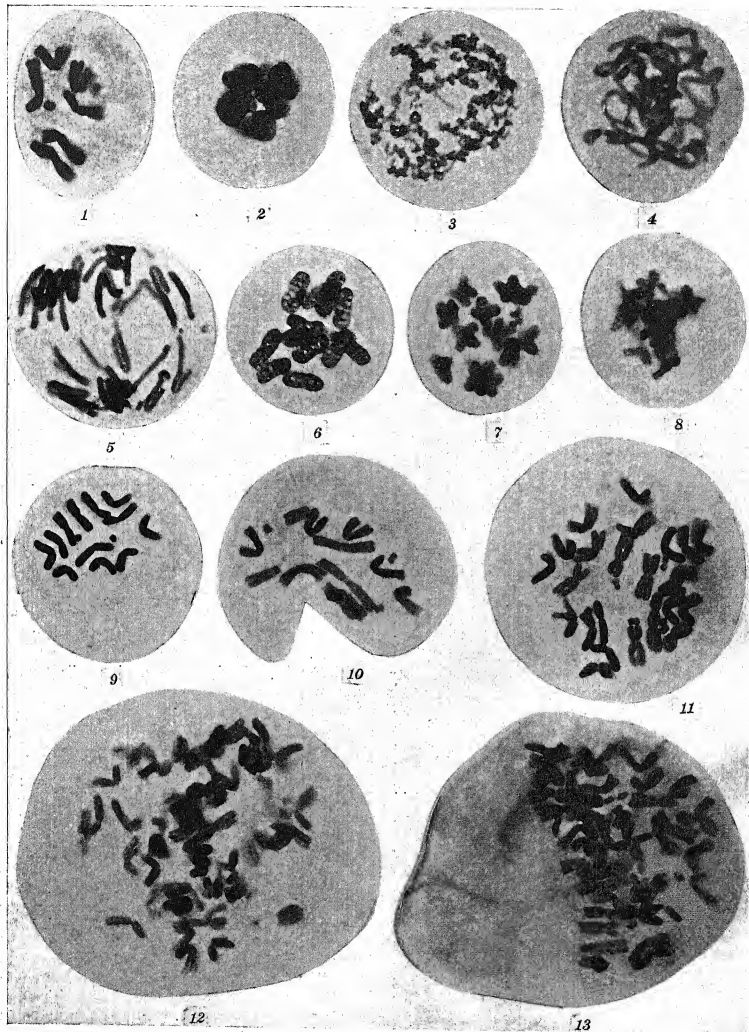


Plate 1, fig. 1-13. Photographs of microspore mother cells and microspores of *Tradescantia* treated with colchicine. The acetocarmine preparations were photographed at magnification of $\times 1200$.—Fig. 1. Normal haploid microspore.—Fig. 2. Microspore mother cell contracted metaphase, one day after treatment.—Fig. 3, 4. Interphase, 3 days after treatment.—Fig. 5. Equational division of twelve univalents, 4 days after treatment.—Fig. 6. Meta-

4, 7, 8, 9, 10). The position of the daughter nuclei (fig. 4, 5) would indicate that they migrate toward each other and fuse (fig. 6, 9). However, since nuclear migrations were not observed, figures 6 and 9 may represent an arrested division of the nucleus. These cells rapidly increase in size, as shown by text figures 1 to 6, and function as diploid microspores.

Microspore mother cells were observed containing twelve univalent chromosomes with no evidence of a two-parted condition (pl. 1, fig. 6). These are associated with cells in the same locale in a resting condition and with cells containing twelve univalents definitely two-parted. It is assumed that in this case the separation of halves of chromosomes does not occur and that the chromosomes reorganize to form a resting nucleus of the diploid microspores. Matsuura (1937) figures similar instances of desynapsis by the effect of heat on the microspore mother cells of *Trilium kamtschaticum*. The nuclei of all diploid microspores, however formed, divide normally (pl. 1, fig. 9, 10) and form the generative and tube nuclei of the developing microgametophyte (fig. 11).

Studies were made of the diploid microspores formed by an equational division of twelve univalents to determine whether colchicine had any effect on nuclear differentiation in microspore development. It was found that nuclear differentiation is normal and that the microspore nucleus divides across the short axis of the cell to form the generative and the tube nuclei. Apparently, colchicine does not affect the synchronization of the nuclear and cytoplasmic activities in the microspores resulting from suppressed meiosis.

Tetraploid microspores (pl. 1, fig. 11), similar to those produced by temperature variation in *Rhoeo* (Sax, 1937), are abundantly formed in material treated with 0.05 per cent colchicine for forty-eight hours and less frequently in material treated with 0.1 per cent for twenty-four hours. Preparations made from material two to nine days after treatment showed that the first meiotic division is suppressed. Twelve bi-partite chromosomes are scattered throughout the cell (pl. 1, fig. 7). These gradually form an irregular mass (pl. 1, fig. 8). It is assumed that the second meiotic division also is suppressed to form the nucleus of the tetraploid microspore. Binucleate cells in which cytokinesis had been suppressed were observed. These are similar to those described for diploid microspores except for an increased size of cell and nucleus. It is possible that in these instances the first meiotic division has already occurred; and the second division has been suppressed. One four-nucleate cell was seen (fig. 12) in which both cell divisions had been suppressed. The major factor in bringing about tetraploidy in this case is the failure of the spindle mechanism to initiate cell division.

Abnormal nuclear divisions are observed in material three to five days after treatment with 0.1 and 0.05 per cent colchicine for twenty-four hours and eight days after treatment with 0.05 per cent for forty-eight hours. Lagging chromosomes, non-disjunction, and multipolar spindles (fig. 13) are observed. Non-disjunction results in unequal chromosome masses at the poles. Triads, pentads, hexads, and octads (fig. 14) are formed. Associated with these cells are ones in which the chromatic material has a diffuse granular appearance. It is assumed that the latter may recover and form diploid or tetraploid microspores.

The individual flowers of *Tradescantia* also show definite response to the colchicine treatment. The pedicels are about one fifth their normal length, the sepals and petals are reduced in size, and the petals are lacking in color. Microscopical examination of cross sections of flower buds show that some of the cells of pedicel and pistil contain large granular polyploid nuclei. A more detailed study of these tissues is planned to determine whether the above mentioned changes in the flower parts are due entirely to chromosome doubling or are in part physiological response to the colchicine.

The first series of cuttings treated with colchicine were planted in moist sand. The majority of these were placed in 0.01 per cent of indole-3-acetic acid² for twenty-four hours before planting to stimulate root formation. Cuttings treated with colchicine alone showed no root development, while those treated with both colchicine and indole-3-acetic acid produced abundant roots. Root smears showed the normal chromosome number. Pollen smears made from plants twenty-four hours after treatment with indole-3-acetic acid showed octoploid pollen grains (pl. 1, fig. 12). One pollen grain which appeared to be hexaploid was observed (pl. 1, fig. 13). The octoploid pollen grains are found in material twenty-one days after treatment with colchicine and one day after treatment with indole-3-acetic acid. It is assumed that they may be formed by suppression of the last premeiotic division and of both meiotic divisions of the microspore mother cell. It is possible, however, that they may be formed by the suppression of the meiotic divisions in the microspore mother cell and of the first division of the microspore.

It is difficult to determine the exact nature of the effect of colchicine on *Tradescantia* inasmuch as all flowers of an inflorescence, or all anthers of the same flower, are frequently not affected equally. Normal haploid pollen grains are often found in locules with diploid pollen grains. It was observed that fewer flowers are affected with concentrations of 0.05 per cent solutions for twenty-four hours than with 0.1 per

²Obtained through the courtesy of Prof. K. V. Thimann, Harvard University.

phase, desynaptic type, 4 days after treatment.—Fig. 7. Twelve bipartite univalents, 3-4 days after treatment.—Fig. 8. Clumped univalents, 4 days after treatment.—Fig. 9. Diploid microspores, 10 days after treatment.—Fig. 10. Abnormal diploid microspore, 11 days after treatment.—Fig. 11. Tetraploid microspore, 12 days after treatment.—Fig. 12. Octoploid microspore, 21 days after treatment.—Fig. 13. Hexaploid microspore, 18 days after treatment.

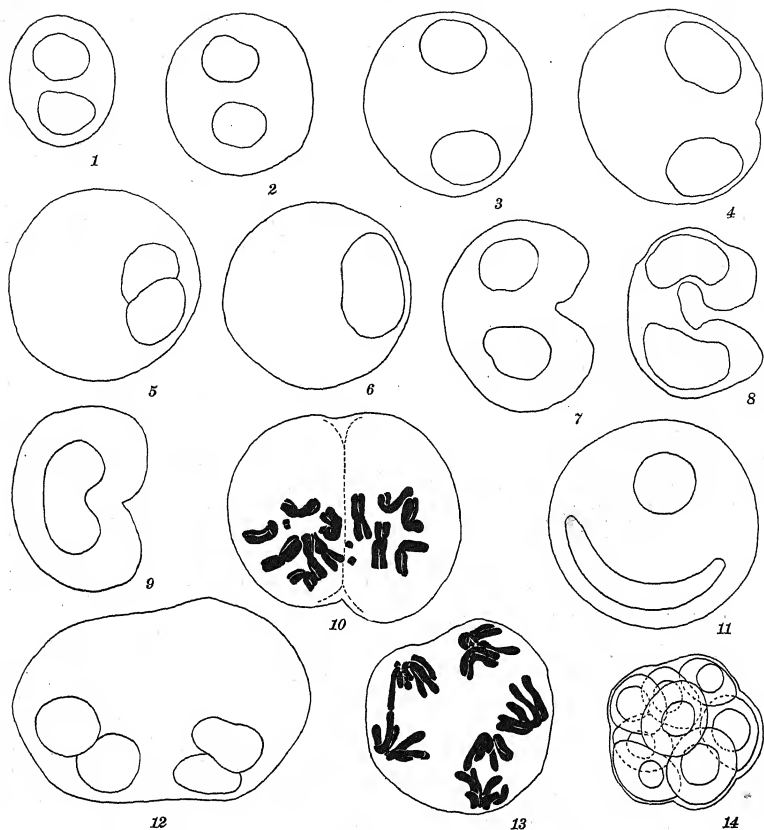


Fig. 1-14 were drawn by the aid of a camera lucida. Magnification $\times 1000$.—Fig. 1-6. Binucleate microspore mother cells, cell division suppressed 4-7 days after treatment.—Fig. 7-9. Binucleate microspore mother cells, cell division initiated later suppressed, 4-7 days after treatment.—Fig. 10. Metaphase of diploid microspore, 11 days after treatment.—Fig. 11. Mature pollen grain, 16 days after treatment.—Fig. 12. Four-nucleate microspore, 7 days after treatment.—Fig. 13. Multipolar spindle, 4 days after treatment.—Fig. 14. Octad, 5 days after treatment.

cent solutions for twenty-four hours and 0.05 per cent solutions for forty-eight hours. A comparison of time required for the development of pollen grains in untreated plants (Sax and Edmonds, 1933) and in treated plants show that meiosis is retarded longer in solutions with higher concentrations. This is in agreement with results obtained by Brues and Cohen (1936) in regenerating tissue of liver.

The suppression of nuclear division in the microspore mother cells appears to be caused by the effect of the colchicine on the cytoplasm. The spindle mechanism may be suppressed during one, two, or more division cycles of the chromosomes. The effect of the colchicine apparently is temporary, as normal spindle formation is resumed in the diploid and tetraploid microspores. Colchicine does not seem to have

any visible effect upon the chromatic material. The chromosomes appear normal at all times. It does, however, retard the chromosome cycle.

There is some evidence that the spindle mechanism is more easily disturbed in meiosis than in mitosis. The colchicine in some instances may induce chromosome doubling in every microspore mother cell in a locule, while polyploid cells in the ovule are sporadic. Contracted metaphases were observed in microspores one day after treatment with colchicine. The effect of the colchicine is temporary, as the cells apparently recover, and division and separation of the chromosomes proceed normally. Colchicine treatment of germinating seeds has not yet produced polyploid plants in the preliminary experiments conducted by other investigators in this laboratory.

SUMMARY

Cuttings of flowering stalks of *Tradescantia* were placed in 0.2, 0.1, 0.05, 0.025, and 0.01 per cent aque-

ous solutions of colchicine for twenty-four hours and in 0.05 per cent for forty-eight hours. Concentrations of 0.025 and 0.01 per cent showed no visible effects on the microspore mother cells and microspores. Concentrations of 0.2, 0.1, and .05 per cent affect the cytoplasm of the microspore mother cells by suppressing the spindle mechanism entirely during one or both meiotic divisions or by inhibiting the formation of cell walls after the occurrence of the first meiotic division. Diploid and tetraploid microspores and pollen grains are produced. Octoploid and hexaploid microspores were occasionally found. The formation of octoploid microspores is attributed to the suppression of both meiotic divisions and either the premeiotic or post-meiotic mitosis. Partial suppression of the spindle mechanism results in the formation of triads, pentads, hexads, and octads.

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EFFECT OF FUNGI ON THE OXIDATION-REDUCTION POTENTIALS OF LIQUID CULTURE MEDIA¹

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BIOLOGICAL OXIDATIONS and reductions which deal with the energy relationships essential to all forms of life have been studied by many investigators, all aiming at an ultimate understanding of these complex processes. Since the release or building up of energy involves the transfer of electrons, it is possible to measure these phenomena in biological systems by oxidation-reduction indicators or electrical apparatus in which electron transfers result in color changes or in the building up of an electrical charge at suitable electrodes.

Investigations on the effects of plants on oxidation-reduction potentials (Eh) have been confined chiefly to bacteria and yeasts. Hewitt (1933) has given an extensive review of the effects of bacteria on electrode potentials, listing over 65 references dealing with this subject. Many more results have been reported on oxidation-reduction phenomena in relation to bacterial metabolism since Hewitt's report was published.

Potter (1912) showed that yeast cells produced reducing conditions in their surrounding medium. Using both indicators and electrical methods of measuring Eh, Cannan, Cohen, and Clark (1926) followed the potential changes produced by yeast cells suspended in buffer solutions. They concluded that yeast cells produce "hydrogen donors" which reduce the media and result in the lowering of the oxidation-reduction potentials. Auel, Aubertin, and Genevois (1929) showed that anaerobic yeast cultures had an Eh of about 160 to 200 millivolts. In a study of the relation of metabolic processes to the oxidation-reduction potentials, Kluver and Hoogerheide (1934) found that anaerobic fermentation began at an Eh of about 90 millivolts while aerobic fermentation began at a potential of about 160-250 millivolts. They found it possible to determine the dominant type of metabolism (i.e., respiration or fermentation) carried on in a yeast culture by observing the oxidation-reduction potential.

In a recent report by Tang and Lin (1936) the results are given of an investigation on the effects of the unicellular algae *Chlorella vulgaris* and *C. pyrenoidosa* on the oxidation-reduction potentials and pH of a synthetic nutrient solution. As shown with bacteria and yeasts, these algae also lowered the Eh of the media when respiration was the dominant type of metabolism—i.e., when the potentials were measured in darkness. In the presence of light, when presumably photosynthesis was the dominant process, the Eh values became more positive.

In an attempt to determine the reason for differences between plants using different forms of nitrogen, Robbins (1937) measured the redox potentials of synthetic media in which *Aspergillus niger* reduced methylene blue, which is reduced at potentials lower than Eh 0.011 at pH 7.0, and that *Rhizopus nigricans* partially reduced 1 naphthol 2 sulfonate indophenol, which is reduced at potentials lower than Eh 0.123 at pH 7.0. He also found that *A. niger* would grow in solutions poised by indicators covering a range of Eh from 0.011 to 0.430 volt.

This paper presents the results of a study of the effects of several filamentous fungi on the oxidation-reduction potentials of liquid culture media.

MATERIALS AND METHODS.—The organisms² used were: Two strains each of *Aspergillus niger* v. Teig, *Sclerotinia fructicola* (Wint.) Rhem. (*Monilinia fructicola* Honey) and one strain each of *Penicillium expansum* Link and *Botrytis Allii* Munn.

Since biological systems generally have low electrical capacities, it is important to draw as little electrical current from them as possible if reliable determinations are to be made. With ordinary potentiometric methods biological half-cells often become polarized during the process of potential measurements and accurate determinations are impossible. A vacuum tube potentiometer was constructed for making Eh determinations. This apparatus made possible such measurements without drawing an appreciable electrical current from the system concerned. In figure 1 is given a diagram of the essential parts of the vacuum tube potentiometer used in this study. Operation of this apparatus is simple and as many as 70 determinations may be made in one-half hour. It is possible to measure differences in potentials of 1 millivolt. No shielding of the vacuum tube is necessary. The vacuum tube potentiometer circuit shown in figure 1 is similar to the circuit described by Partridge (1929).

To determine the voltage at the platinum electrode or glass electrode, both of which dip into the liquid in the culture flask as shown in figure 1, the procedure is as follows: With switch S3 in lower position, switch S2 is closed and the vacuum tube allowed to warm up. Switch S1 is thrown to the right and S3 placed in the upper position. With S1 in the right position the negative terminal of the calomel half-cell/experimental half-cell system is connected to the grid of the vacuum tube. The potential of the current flowing from the cathode of the vacuum tube

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This work was carried on in the Laboratory of Plant Physiology at the University of Wisconsin.

The writer wishes to express his appreciation to Dr. B. M. Duggar for his interest and many helpful suggestions during the progress of the work and the preparation of the manuscript.

² The cultures of these organisms were secured through the courtesy of the following individuals: *Aspergillus niger* No. 3, *Penicillium granulosum* No. 1, and *P. granulosum* No. 4641 were obtained from H. C. Green. *Aspergillus niger* No. 1, *Penicillium expansum*, and *Botrytis Allii* were supplied by K. M. Baker. *Sclerotinia fructicola* W 60b and W129 were obtained from E. E. Honey.

to the screen grid is balanced against a reference potential governed by the fixed resistance of R1. Balancing is accomplished by adjusting the voltage on the cathode by means of the variable resistance of R1. When the potential of the current flowing through the electron tube is equal to that of the reference potential, there will be no deflection in the galvanometer (G). The switch S1 is then thrown to the left, connecting the grid to the negative terminal of the potentiometer. Enough voltage is applied to the grid by the potentiometer to again balance the two circuits described above. The amount of voltage applied to the grid of the vacuum tube by the potentiometer is the same as was being supplied by the two half-cells. When the above determination is made with the platinum electrode, the voltage may be corrected to the potential of the normal hydrogen electrode since the voltage of the calomel half-cell is referred to the hydrogen electrode. The resulting corrected voltage is the Eh of the liquid in the culture flask.

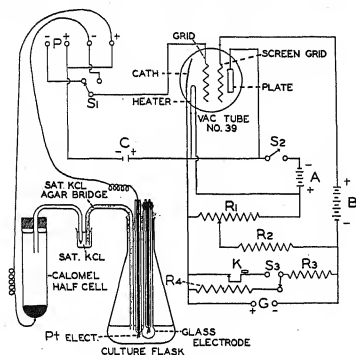


Fig. 1. Vacuum tube potentiometer and electrode assembly. Vacuum tube, National Union vacuum tube No. 239 (39/44). A, 6 volt storage battery; B, 22½ volt "B" battery; C, 1½ volt dry cell battery; G, galvanometer (Leeds and Northrup, Cat. No. 2330-D); K, tapping key; P, potentiometer (Pyroelectric Instrument Co. direct reading "Millivolt," Serial No. 378); R1, 32 ohm fixed and variable resistance; R2, 10,000 ohm fixed resistance; R3, 1 ohm fixed resistance; R4, 30 ohm fixed resistance; S1, S2, and S3, switches.

Bright platinum electrodes were used for determinations of oxidation-reduction potentials in the fungous culture media. Hydrogen-ion concentrations in the media were measured with the quinhydrone electrode or glass electrodes. The glass electrodes used were of the Haber and Klemensiewicz (1909) bulb type employing quinhydrone and 0.10M HCl and bright platinum electrodes. When pH measurements were made with glass electrodes, the vacuum tube potentiometer was used, no modifications being necessary.

Stock cultures of the fungi studied were maintained on test tube slants of potato dextrose agar. This medium proved to be satisfactory for spore production by these fungi.

Erlenmeyer flasks of 125 cc. capacity were used as culture vessels in the following experiments. To each flask was added 35 cc. of the desired culture medium. These flasks with contents were sterilized by autoclaving for 20 minutes at 15 pounds pressure.

The apparatus for electrolytic measurements of the culture medium was assembled as follows: Groups of 2 platinum electrodes, a U-tube of 3 mm. glass tubing for the KCl agar bridge, and where pH was to be measured with glass electrodes, a glass electrode (unfilled), were wrapped in cotton plugs and inserted into 125 cc. Erlenmeyer flasks containing enough distilled water to cover the metal tips of the platinum electrodes. These assemblies were sterilized in the same manner as the flasks containing the culture medium. Following sterilization, the U-tube described above was filled with 2 per cent agar dissolved in a solution of saturated KCl.

Using young, sporulating stock cultures, heavy spore suspensions were prepared of the desired fungi. A few cc. of sterile, physiological salt solution were added to the test tube stock cultures and the spores suspended by scraping the surfaces with a sterile loop. The spore suspensions of the *Penicillium* strains were difficult to maintain unless used immediately, while those of the other organisms were readily made and fairly permanent.

The sterile medium in each of the culture flasks was inoculated with 0.5 cc. samples of the above spore suspensions. This quantity of inoculum was found to be sufficient to exclude spore load as a limiting factor in the resulting cultures.

In each inoculated flask was placed a cotton plug containing a sterile electrode assembly and an agar bridge. The electrodes and the agar bridge dipped into the culture medium, as is shown in figure 1.

The cultures were set up in triplicate with each fungus for all media studied. The cultures were arranged in groups of six around a reservoir containing a saturated solution of KCl into which dipped the exposed ends of the agar bridges. The side arm of the calomel half-cell also dipped into this reservoir to complete the circuit between the two half-cells.

Three flasks containing sterile medium, but otherwise identical to the inoculated cultures containing electrodes, served as controls for each medium used.

The cultures were incubated in a room in which the temperature was maintained at 25°C. All measurements of Eh and pH were made at this temperature.

Dry weight determinations were made of the fungous mats produced by the several strains of fungi on all media in which Eh and pH measurements were made. After the last electrode reading had been taken the fungous mats were removed from the flasks and placed on previously weighed, oven-dried filter paper. The mats were washed several times with

distilled water and then dried to a constant weight in an oven held at a temperature of 100°C.

RESULTS.—*Experiment 1.*—Cultures of *Sclerotinia fructicola* W60b, *S. fructicola* W129, *Aspergillus niger* 3, *Penicillium expansum*, and *Botrytis Allii* were set up, using a buffered prune extract medium.

This medium was prepared by cooking 100 grams of dried Italian prunes in 500 cc. of distilled water for 2 hours in an Arnold steamer. The cooked prunes were then broken up and the mass allowed to stand for 1 hour. The solid material was removed by filtering, and the resulting filtrate was made up to a volume of 1000 cc. Ten grams of KH_2PO_4 were added to the liter of extract to increase the buffering effect.

In figure 2 are shown the effects of these fungi on the oxidation-reduction potentials of this medium. Each point on the curve is an average value of six determinations.

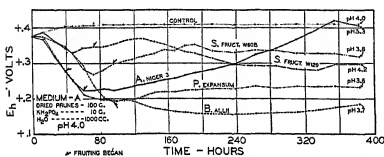


Fig. 2. The effects of *Sclerotinia fructicola* W60b and W129, *Aspergillus niger* 3, *Penicillium expansum*, and *Botrytis Allii* on the oxidation-reduction potentials of a buffered, prune extract medium.

The initial pH of this medium was 4.0 and the Eh about 0.375 volt. The curve for the sterile medium shows that following an initial rise of about 0.025 volt in 40 hours, the potential remained relatively constant. The hydrogen-ion concentration did not change in the sterile medium during the 383-hour period.

As growth began in the cultures there was a decrease in Eh in all cases. *A. niger* and *B. Allii* produced the most rapid drop in potential during the first 60 hours. The other fungi reduced the potential of the medium more slowly. The two strains of *S. fructicola*, while lowering the potential of the medium only about 0.1 volt during the entire period of culture, during the first 48 hours lowered the Eh at about the same rate as *B. Allii*. The latter organism effected the most intense reducing conditions in this medium, reaching a level of Eh 0.161 volt in 240 hours.

The curves for *B. Allii* and *P. expansum* show that, following the initial drop in potentials in the cultures, a relatively constant Eh level was reached. After 240 hours there was little change of potential in the *Sclerotinia* cultures. The behavior of *A. niger* 3 was in contrast with that of the other fungi with this medium. Following the drop to 0.223 volt, the Eh gradually increased until a potential similar to that of the sterile, control medium was attained.

The hydrogen-ion concentrations of the cultures at the time they were taken down are shown at the extreme right in figure 2. It will be noted that the range in pH between the cultures was relatively narrow with this medium. The pH of the medium on which *A. niger* 3 was grown was 3.3, which represents the most acid final hydrogen-ion concentration.

The times at which the first spores appeared on the various cultures are indicated on the curves by checks.

From the appearances of the cultures and on the basis of dry weights of the fungous mats produced (which will be discussed later), this medium is favorable for these fungi. In nearly all cases, surface mat formation was complete in about 4 days after inoculation.

Experiment 2.—Strains 1 and 3 of *A. niger* and 1 and 4641 of *P. granulatum* were grown on a synthetic culture solution. This medium was a modification of Richards' (1897) solution (composition shown in figure 3). Glucose was substituted for sucrose in approximately equimolar concentration, and 10 grams of KH_2PO_4 was added to a liter of the medium instead of the five grams suggested by Richards. The increased amount of KH_2PO_4 was used to increase the buffering power of the medium.

The cultures were set up in the same manner as in the previous experiment. The controls consisted of flasks containing sterile medium. Eh measurements of the cultures and controls were made at intervals during a period of 392 hours after inoculation. The results of the action of these fungi on the Eh and pH of the medium are shown in figure 3.

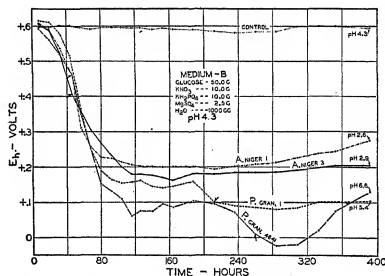


Fig. 3. The effects of *Aspergillus niger* 1 and 3 and *Penicillium granulatum* 1 and 4641 on the oxidation-reduction potentials of a synthetic medium.

The oxidation-reduction potential and hydrogen-ion concentration of the sterile medium remained constant at Eh 0.6 volt and pH 4.3, respectively, during the course of the experiment. There was a rapid drop in the potential of the medium with both strains of *A. niger* and *P. granulatum* during the first 80 hours. Soon after this time the *A. niger* cultures appear to have reached a relatively constant Eh level at about

0.2 volt. The *P. granulatum* cultures continued to lower the potential until at 280 hours *P. granulatum* 4641 attained a level of Eh -0.022 volt. This represents a total drop of over 0.6 volt from the original value.

Hydrogen-ion concentration measurements taken at the end of the culture period showed that the medium of the *Aspergillus* cultures became more acid than the sterile medium, whereas the medium of the *Penicillium* cultures was adjusted toward neutrality. The average pH of the *A. niger* cultures was pH 2.6 for strain 1 and pH 2.9 for strain 3. The average value for the *P. granulatum* cultures as pH 5.4 for strain 1 and pH 6.6 for strain 4641.

This medium was favorable for the growth of the *Aspergillus* and *Penicillium* strains used. Wrinkled surface mats were produced in each instance. The *A. niger* strains produced spores at an Eh level between 0.4 and 0.5 volt in less than 48 hours, while the *P. granulatum* strains sporulated at an Eh level between 0.1 and 0 volt in about 10 days.

Experiment 3.—*Aspergillus niger* 3, *B. Allii*, *P. expansum*, and *P. granulatum* 4641 were grown in a synthetic culture solution having the same constituents as Richards' solution. The only quantitative modification was that 10 grams of KH_2PO_4 were used in place of 5 grams. The data obtained with this medium are presented in figure 4.

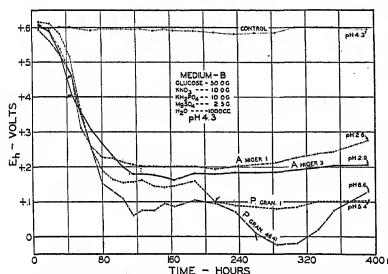


Fig. 4. The effects of *Aspergillus niger* 3, *Botrytis Allii*, *Penicillium expansum*, and *P. granulatum* 4641 on the oxidation-reduction potentials of a synthetic medium.

The initial Eh of the medium was about 0.6 volt and the hydrogen-ion concentration pH 4.3. The potential in the sterile medium of the controls changed from Eh 0.630 to 0.576 volt during a period of 309 hours. The hydrogen-ion concentration remained constant in the sterile medium.

As observed in the two previous experiments, the Eh of the medium was lowered in all cases as growth began in the cultures. *A. niger* 3 produced the most intense reduction of potentials during the first 72 hours after inoculation. This organism reduced the potential from Eh 0.6 volt to about 0.2 volt in about 80 hours and maintained this level with little change during the rest of the culture period.

The most intense reducing conditions were produced in the *P. granulatum* 4641 cultures, the potential dropping continuously to Eh 0.028 volt in 177 hours. This decrease was followed by a rise in potential until, at the end of 309 hours, the Eh of the medium in which this fungus was growing was 0.2 volt. The potentials of the *P. expansum* cultures show a sudden rise in Eh after 36 hours. Until this time the potential was being lowered at about the same rate as in the *A. niger* 3 cultures. At the same time as the sudden rise in potential was observed in the *P. expansum* cultures, the first spores were observed. It is possible that the process of spore formation by this organism had some effect on the Eh of the medium. It is also possible that the decrease in hydrogen-ion concentration was rapid enough at this stage to have caused the shift in potentials in the cultures.

The final Eh levels produced by these fungi on this medium were within a range of 0.075 volt—i.e., between Eh 0.192 and 0.267 volt. There was considerable variation in final pH values, however. *Botrytis allii* and *P. granulatum* gave final pH values similar to that of the control, pH 4.3. *A. niger* 3 adjusted the medium to a more acid condition, pH 3.0, and *P. expansum* changed the medium to pH 6.1.

All the fungi cultured on this medium gave good growth except *Sclerotinia fructicola* W129, which in a preliminary experiment gave no response at all.

Experiment 4.—To 1000 cc. of a medium having the same composition as medium B, which is given in figure 3, was added 0.1 g. of cysteine hydrochloride. It was found that greater concentrations of this chemical increased the acidity of the resulting medium to a range unfavorable for the growth of the fungi studied. The 0.01 per cent concentration of cysteine hydrochloride used in this experiment reduced the Eh of the autoclaved medium from 0.6 volt to about 0.4 volt. The hydrogen-ion concentration was pH 4.0.

Using the above medium, cultures of *A. niger* 3, *P. granulatum* 4641, *B. allii*, and *S. fructicola* W129 were set up. The results of Eh measurements made over a period of 347 hours in these cultures and the sterile medium are given in figure 5.

Very little change in the oxidation-reduction potential of the sterile medium occurred, as is shown by the curve for the controls. The pH of the medium remained constant at pH 4.0.

Aspergillus niger 3 lowered the Eh of the culture medium more rapidly than any of the other organisms employed. A potential of 0.129 volt was reached in 70 hours, following which the Eh increased to a value slightly more than 0.2 volt and remained relatively constant during the remainder of the period of culture.

The curve showing the effect of *B. Allii* on this medium indicates an increase in oxidizing tendency of the medium as growth began. The appearance of these cultures differed in no way from that of cultures of the same organism grown on the other media. Following this rise, the Eh dropped to a level of about

0.2 volt, and this level was maintained during the remainder of the culture period.

The most intense reducing conditions set up in this medium occurred in the *P. granulatum* 4641 cultures, an Eh of -0.014 volt being reached in 310 hours after inoculation. The final determination made of the Eh of these cultures gave a value more than 0.2 volt lower than any of the other cultures at this time.

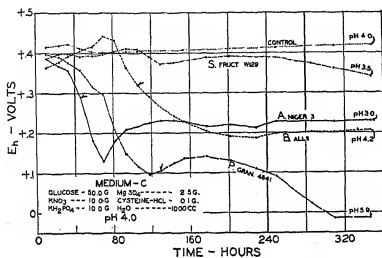


Fig. 5. The effects of *A. niger* 3, *B. Allii*, *P. granulatum* 4641, and *S. fructicola* W129 on the oxidation-reduction potentials of a synthetic medium to which cystein hydrochloride was added.

The culture medium in which *S. fructicola* W129 was grown showed little change in the oxidation-reduction potential. Only a small number of submerged colonies were produced. These colonies did not increase in size after the first few days of culture. At the end of the experiment they were distinctly brownish in appearance.

The *A. niger* 3 culture medium had a hydrogen-ion concentration of pH 3.0 at the close of the experiment, an adjustment of the medium to a more acid reaction during the period of culture. *Botrytis Allii* had little effect on the pH of this medium, while *P. granulatum* 4641 changed the reaction of the medium from pH 4.0 to 5.9.

With the exception of *S. fructicola* W129, these fungi produced good growth on this medium. Spore production occurred in the same order as was observed with the same fungous strains on other media.

Experiment 5.—In an experiment in which it was desired to measure pH continuously as well as Eh, *A. niger* 3, *B. Allii*, and *P. granulatum* 4641 were grown on a medium having the same composition as medium B (see figure 3).

Oxidation-reduction potential determinations were made in the usual manner. The hydrogen-ion determinations of the cultures were made by means of glass electrodes.

One glass electrode was placed in each culture along with the two platinum electrodes. From previous experiments it had been found that sterilization of the empty glass electrode bulbs in the autoclave did not change the characteristics of the electrodes when

they were completely assembled later. Sterilization of the glass electrodes containing 0.1 M hydrochloric acid, quinhydrone, and a platinum electrode made pH determinations unreliable, probably due to decomposition of the quinhydrone by heat.

Each of the three control vessels containing the sterile medium also contained a glass electrode. Since the pH of the controls remained constant during the experiment, changes in the glass electrode characteristics in the fungous cultures could be corrected. All the glass electrodes had the same E/pH characteristics within 8 millivolts at the beginning and end of the experiment. This placed the accuracy of the pH determinations within limits of 0.06 pH, since the glass electrodes used gave 58 millivolts change for each pH unit. Final calibration of the glass electrodes was made with standard buffer solutions at pH 3.98, 6.0, and 7.0 at the time the cultures were taken down.

The initial Eh of the medium was about 0.63 volt and the hydrogen-ion concentration, pH 4.3. The period of culture continued until the fungi began to sporulate.

Figure 6 gives the results of the determinations of Eh and pH on the above cultures.

The Eh curves for these organisms are similar to those obtained for medium B. *Aspergillus niger* 3 again produced the most intense reducing conditions in the medium for the first period of growth, reaching a level of about Eh 0.2 volt in 140 hours. *Botrytis Allii* required more time to reach the same intensity of reduction of the medium but reached almost the same final Eh at the end of the experiment.

The pH curves in the lower graph of figure 6 show a decrease in hydrogen-ion concentration in the cul-

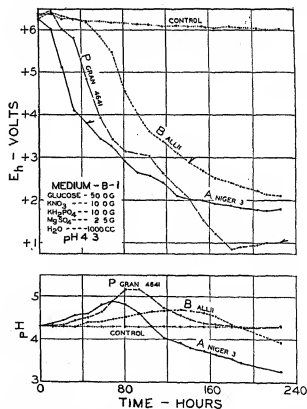


Fig. 6. The effects of *A. niger* 3, *B. Allii*, and *P. granulatum* 4641 on the oxidation-reduction potentials and hydrogen-ion concentration of a synthetic medium.

ture media of the three fungi during the initial growth period. *Aspergillus niger* 3 changed the reaction of the medium from pH 4.3 to pH 4.9 in 69 hours. Following this decrease, the hydrogen-ion concentration increased in the cultures of this organism until at 226 hours the pH was 3.25. As will be shown later, the decrease in pH in this medium opposes decrease in Eh. Thus *A. niger* 3 was capable of maintaining and even reducing the oxidation-reduction potential of the medium even though there was an increase in hydrogen-ion concentration.

The decrease in acidity of the *P. granatum* 4641 culture medium continued for 81 hours until a value of pH 5.17 was reached. After this time the hydrogen-ion concentration increased, reaching pH 4.27 in 190 hours after inoculation of the medium. The final pH value for this fungus was pH 4.33. In general, this organism produced results similar to those observed for *A. niger* 3 in regard to both the Eh and pH changes except that *P. granatum* 4641 reduced the medium to a lower Eh level and *A. niger* 3 produced a more acid reaction in the medium by the end of the experiment.

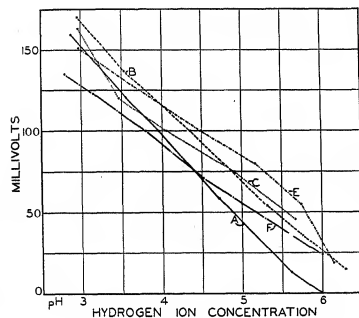


Fig. 7. The E/pH relationships in the media used.

The culture medium on which *B. Allii* was grown showed comparatively small changes in pH, while the Eh decreased in the usual manner from over Eh 0.6 volt to about 0.2 volt. The gradual increase in pH continued for 130 hours to produce a reaction of pH 4.70. The final hydrogen-ion concentration of the media on which *B. Allii* was cultured was pH 3.93.

Experiment C.—In order to determine the relative effects of changes in pH on the Eh of the sterile nutrient solutions used in the preceding experiments, samples of these solutions were adjusted to various hydrogen-ion concentrations by adding various amounts of 0.5 M hydrochloric acid and potassium hydroxide. Hydrogen-ion concentrations were determined by means of glass electrodes and Eh measurements were made in the usual manner with platinum electrodes. The range of pH considered in these determinations

was in most cases from about pH 3.0 to 6.0, since this range covered the change in reaction of the media brought about by the growth of the fungi studied. All determinations were made at 25 degrees C. The results of these determinations are presented in figure 7.

For convenience in comparing the effects of an alteration of hydrogen-ion concentration on the Eh of the various media used, the curves are presented in one graph and the corrected oxidation-reduction values are not given for any of the solutions. The letters appearing on the curves identify the media previously referred to in figures 2 to 6.

Curve B shows that the E/pH relationship in the glucose- KNO_3 - KH_2PO_4 - MgSO_4 medium was about 45 millivolts for a change of one pH unit. Curve C is for the glucose- KNO_3 - KH_2PO_4 - MgSO_4 -cysteine hydrochloride medium and indicates an average slope of about 42 millivolts for each pH unit. The E/pH relationship of the sucrose- KNO_3 - KH_2PO_4 - MgSO_4 medium shown in curve E sets the change in voltage for one pH unit at about 38 millivolts. Curve F shows the above relationship for a synthetic medium³ consisting of 50 g. glucose, 7 g. $\text{Ca}(\text{NO}_3)_2$, 10 g. KH_2PO_4 , 25 g. MgSO_4 , and 1000 cc. H_2O . This curve indicates an average change of 38 millivolts for each pH unit.

These curves show that the hydrogen-ion concentration of the media employed had a definite effect on the oxidation-reduction potentials. The relationship varied from 38 to 55 millivolts in Eh for a change of pH of one unit in the range of hydrogen-ion concentration considered. The average change in potential at the platinum electrodes for the five culture solutions for a change in pH of one unit was 45 millivolts.

Pinckard (1935) gives a graph showing the relation between oxidation-reduction intensity and hydrogen-ion concentration in a "yeast-extract-mineral-salts-glucose" medium. It was found that a shift in potential of about 48 millivolts resulted from each change of one pH unit in a hydrogen-ion concentration range from pH 2 to pH 11. In a theoretical discussion of the possible effects of hydrogen-ion concentration on oxidation-reduction potentials of reversible oxidation-reduction systems, Clark and Cohen (1923) state that for a change in pH of one unit there may be shifts in potentials of zero, 30, 60, or 90 millivolts, depending upon the system concerned.

The curves in figure 7 are not to be considered Eh/pH curves for true reversible oxidation-reduction systems. They do indicate, however, to what extent the Eh values in the preceding experiments might have been affected by H-ion changes in the cultures.

Fungous mats.—The average dry weights of the fungous mats produced by the various fungi on the nutrient media used in the foregoing experiments as well as on medium F are given in table 1.

³ The fungi (*A. niger* 3, *P. expansum*, *P. granatum* 4641 & 1, and *B. Allii*) grown on this medium gave similar reactions as shown for medium E regarding the Eh and pH changes produced.

TABLE 1. Dry weights of fungous mats (in grams) produced in the various culture media.

Fungus	Culture medium					
	A	B	C	B-1	E	F
<i>A. niger</i> no. 3	2698 ± .014 ^b	4444 ± .022	3015 ± .015	3885 ± .019	2788 ± .014	5279 ± .026
<i>P. expansum</i>1615 ± .008				2789 ± .014	3304 ± .017
<i>P. granulatum</i> no. 4641 ..		4053 ± .020	4955 ± .025	4075 ± .020	3098 ± .016	5897 ± .029
<i>P. granulatum</i> no. 1		3053 ± .015				4011 ± .020
<i>B. Allii</i>	2882 ± .014		4506 ± .023	2291 ± .011	3268 ± .016	4337 ± .022
<i>S. fructicola</i> W60b1979 ± .010					
<i>S. fructicola</i> W1296652 ± .033		.0171 ± .001			
Length of culture period in hours	383	356	347	226	309	359

^a Average of three cultures.^b Probable errors calculated by the "Deviation from the Mean" method.

Since the length of the growing period varied in the several experiments, exact comparisons of the growth response of the organisms to the media could not be made. The dry weights given in table 1 do show, however, the suitability of the media for the organisms employed and the differences in response between the fungi grown on the same medium.

Sclerotinia fructicola W129 gave the heaviest growth on the prune extract medium, the average weight of the mats of this fungus being more than twice the weights of mats produced by *A. niger* 3 or *B. Allii*. It is of interest to note that the average weight of the *S. fructicola* W60b mats was only one-third that of the *S. fructicola* W129 cultures.

Of the synthetic media, F proved to be most favorable on the basis of dry weights of fungous mats produced. Whether this favorable response was due to the presence of calcium in the medium or to its effect on the hydrogen-ion concentration was not determined. With the exceptions of the *S. fructicola* strains, the synthetic media gave heavier mats for the fungi studied than medium A. The higher available carbohydrate content of the synthetic media probably accounts for the increased dry-weight-of-mat yield from the fungi grown on them in comparison to the prune extract medium A.

The two strains of *S. fructicola* failed to give an appreciable amount of growth on any of the synthetic media used. The only case where sufficient growth appeared for dry weight determinations to be made was in medium C to which was added cysteine hydrochloride.

Discussion.—The relative effects of *A. niger* 3, *P. granulatum* 4641, and *B. Allii* on the oxidation-reduction potentials of the media employed are shown separately in figures 8, 9, and 10, respectively. These three fungi were grown on nearly all the media previously described. The letters on the curves refer to the various culture solutions of the preceding experiments.

The curves given in figure 8 show the changes in Eh produced by *A. niger* in six media.

In all cases this fungus lowered the Eh levels of the media as growth began. The period of intense

lowering of the electrode potentials in the media corresponds rather closely with the time during which the fungus was growing rapidly and its hyphae were spreading throughout the medium. An Eh of about

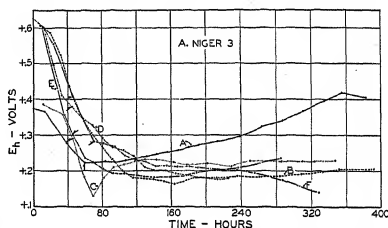


Fig. 8. Effects of *Aspergillus niger* 3 on the oxidation-reduction potentials of the media in which it was grown.

0.2 volt was reached in 120 hours after inoculation of *A. niger* 3 on all media. With the exception of the prune extract medium (A), a reduction potential of about Eh 0.2 volt was maintained during the rest of the periods of culture. The reduction of the potentials of the synthetic culture media to this Eh level appeared to be characteristic of *A. niger* 3. By the time the Eh level of approximately 0.2 volt was reached in the cultures of this fungus, there was considerable fruiting and evidences of autolysis appeared.

The cause of the drift towards more oxidizing conditions in medium A after 96 hours was not determined. The change in hydrogen-ion concentration from the initial pH of 4.0 to pH 3.5 was not sufficient to account for the change unless the reaction changed to very alkaline conditions by 96 hours after inoculation and then shifted back to the final acidity.

Following the log phase of growth, certain bacteria produce an increase in Eh (Hewitt, 1933, and others). This behavior was particularly characteristic of many anaerobic bacteria producing hydrogen peroxide and no catalase.

In figure 9 are given the Eh-time curves for the five media on which *P. granulatum* 4641 was grown.

It is evident that this organism does not behave consistently in the media used, with respect to the oxidation-reduction potential reached in a given time. As was noted in the *A. niger* 3 cultures, *P. granulatum* 4641 reduced the Eh of the media in which it was grown rapidly during the phase of the culture period in which the vegetative hyphae were spreading throughout the media. In few instances were the potentials of the media maintained at constant Eh levels for more than a few hours. The cultures set up with medium F gave the least reduction in Eh during the culture period. The most intense reduction potentials produced by this fungus occurred in media B and C. In both cases a slightly negative Eh value was measured at the electrodes near the close of the culture periods. The Eh of -0.024 volt in medium B was the lowest value reached by any of the fungi used.

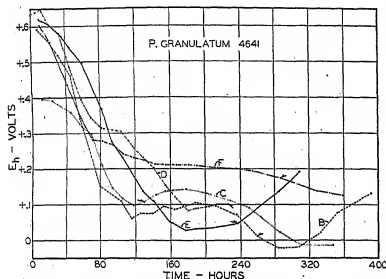


Fig. 9. Effects of *Penicillium granulatum* 4641 on the oxidation-reduction potentials of the media in which it was grown.

Figure 10 is a composite graph showing the oxidation-reduction potential changes that *B. Allii* brought about in the media on which it was grown. These Eh curves indicate that this organism behaved in a manner similar to *A. niger* 3 regarding the potential changes in the media. There was a lag, however, in the *B. Allii* cultures before intense reduction began.

As was noted in the cultures of the other fungi studied, the most rapid drop in Eh occurred in *B. Allii* cultures during the first part of the culture period. An exception to this behavior may be observed when this fungus was grown on medium C to which cysteine hydrochloride had been added. Here the Eh increased for a period of about 72 hours. As previously mentioned, this compound is readily oxidized and its oxidation by *B. Allii* during the initial growth period probably accounts for the rise in potential. Following the increase in potential, reduction occurred at a similar rate as in the other media and a similar final Eh level was reached.

Botrytis Allii appeared to be able to reduce the Eh of the media used to a value of about 0.2 volt. After 160 hours more or less stable potentials were established in these media except in medium F where reduction of Eh continued for about 280 hours.

Penicillium expansum was grown on two synthetic nutrient solutions (media E and F) and on the prune extract medium. This organism produced spores on these media in about 40 hours after they were inoculated. In the case of medium E, spore formation coincided with a rapid rise in potential. Whether the increase in Eh was the result of spore production or not was not determined. In neither of the other media (A and F) were there indications of potential change at the time of fruiting. The lowest potential produced by *P. expansum* on the media employed was Eh 0.046 volt in medium F 350 hours after inoculation of the cultures.

The strains of *S. fructicola* (W60b and W129) studied had little effect on the oxidation-reduction potentials of the medium on which they were successfully grown, medium A. The two strains produced similar effects on this medium, reducing it slowly to an Eh of about 0.3 volt from an initial value of Eh 0.4 volt. Attempts to grow these fungi on the synthetic media were unsuccessful.

The fungi *A. niger* 1 and *P. granulatum* 1 produced similar effects on the media as have already been discussed for *A. niger* 3 and *P. granulatum* 4641. *Penicillium granulatum* cultures 1 and 4641 on media B and F had similar final Eh values, the differences being less than 0.06 volt in both cases. The Eh changes recorded for *A. niger* 1 cultures very closely paralleled the values obtained for *A. niger* 3 cultures on medium B, except that toward the end of the period of culture strain 1 cultures showed a stronger tendency to produce oxidizing conditions in the medium. The final Eh value for *A. niger* 1 was 0.275 volt.

Robbins' (1937) found the intensity of reducing conditions established in a synthetic medium by *Aspergillus niger* and *Rhizopus nigricans* to be about Eh 0.011 and 0.123, respectively, at pH 7.0. The results reported here indicate a similar general reducing level for the fungi and media studied. The lowest Eh value recorded by either *A. niger* strain was

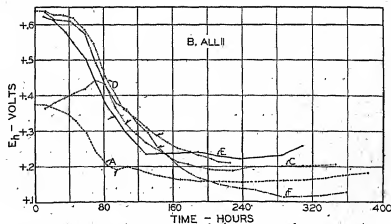


Fig. 10. Effects of *Botrytis Allii* on the oxidation-reduction potentials of the media in which it was grown.

with medium C where *A. niger* 3 reached a value of 0.129 volt 70 hours after the cultures were set up. The difference between maximum reducing intensity obtained by Robbins and the writer for *A. niger* might well be accounted for in variance in methods of culture, the strains of *A. niger* used, or the pH at which potential measurements were made.

The changes in oxidation-reduction potentials observed in the media in which these fungi were grown are in general similar to changes effected by certain bacteria and yeasts on their culture media. With the latter organisms, more or less rapid reduction of the Eh levels in the media occurs during the log phase of growth, depending on the organism, medium, and the conditions under which measurements are taken. The period during which the fungi used were growing rapidly and filling the media with vegetative mycelia might be compared with the log phase of growth in bacterial and yeast cultures. It was during this active, vegetative growth period that the most intense reduction of the Eh level took place in the fungous cultures.

The intensity of reducing conditions established in the media by the fungi studied is similar to the Eh value reported by Kluyver and Hoogerheide (1934) for *Saccharomyces cerevisiae* grown in air in a glucose-phosphate medium. This organism reduced the potential of the medium to Eh 0.22 volt. Lower values were obtained with this yeast when grown in the same medium in the presence of nitrogen. Cannan, et al. (1926) report reducing intensities of Eh -0.20 volt being reached in anaerobic yeast cultures where the medium was a buffer solution of pH 9.25. They also found that when oxidized glutathione was added to the culture medium the yeast cells lowered the Eh from 0.27 volt to -0.07 volt.

For the range in Eh levels of the media studied—i.e., from Eh 0.4 to 0.6 volt—there appeared to be little difference in the ability of these fungi to initiate growth. Variation in the initial Eh of the media did not have an apparent effect on the final Eh level reached in the cultures.

The data indicate that the process of spore formation by the fungi used was not dependent upon the oxidation-reduction potentials of the media. *Aspergillus niger* 3 produced spores over a range of Eh 0.40 to 0.20 volt, *P. granulatum* 4641 over a range from Eh 0.18 to 0.1 volt, *B. Allii* over a range from Eh 0.34 to 0.19 volt, and *P. expansum* over a range from Eh 0.54 to 0.28 volt. The time and Eh at which sporulation took place apparently depended on the fungus and the medium employed.

The relationships between Eh and pH in the culture media in which the organisms were present was undoubtedly more complex than the relationships determined by titration of the sterile media with potassium hydroxide and hydrochloric acid as shown in figure 7. With this procedure the change in Eh for each change in pH varied from 38 millivolts for media E and F to 55 millivolts for medium A. Thus if the change in reaction of the fungous cultures were ac-

complished by the production of such simple acids or bases as above, the change in Eh of the cultures would be affected by less than 100 millivolts in all cases. The greatest change in reaction of a set of cultures occurred when *P. granulatum* 4641 was grown on medium B where the pH changed from an original reaction of pH 4.3 to pH 6.59 at the close of the period of culture.

That the Eh values of the cultures were not the direct result of changes in hydrogen-ion concentration is shown in figure 6 in which both Eh and pH changes taking place in the cultures were recorded. In each case the Eh level dropped as the pH value increased, but the lowering of potentials continued even when the cultures began to produce more acid reactions. In the *B. Allii* cultures the change in pH was less than one pH unit when the Eh change was about 400 millivolts.

Of the fungi studied, *B. Allii* had least effect on the hydrogen-ion concentrations of the media. The greatest change which occurred was in medium F where the pH of the medium was pH 3.8 at the beginning and pH 4.6 at the end of the culture period, a change of 0.8 of a pH unit. The *A. niger* 3 cultures produced reactions more acid than those of the sterile media in every case except with medium F which contained $\text{Ca}(\text{NO}_3)_2$ in place of KNO_3 as a nitrogen source. This medium was adjusted from pH 3.8 to pH 5.8 in 539 hours of culture. In no case did the *P. granulatum* cultures produce more acid reactions than the original H-ion concentrations of the media. With 3 of the 5 media on which this fungous was grown the pH changed toward more alkaline reactions.

The nature of the substances produced in the fungous cultures which were responsible for the changes in oxidation-reduction potentials in the media was not determined. It is interesting to note that the Eh changes in the cultures of the fungi were similar to those observed in cultures of certain bacteria and yeasts. The reduction of the Eh in the media of the latter organisms has been variously attributed to the production of "hydrogen donors," respiratory enzymes and pigments, or other substances capable of influencing electrode potentials. The changes in oxidation-reduction potentials in the surrounding media, produced by micro-organisms, are probably the results of the presence of several substances, the exact nature of which remains to be determined.

SUMMARY

The effects of two strains each of *Aspergillus niger*, *Penicillium granulatum*, and *Sclerotinia fructicola*, and one strain each of *Botrytis Allii* and *Penicillium expansum* on the oxidation-reduction potentials of several liquid culture media were determined.

A vacuum tube potentiometer made possible the measurements of electrode potentials without the danger of polarization of the electrodes. With the technique employed it was possible to determine Eh and pH without disturbing the fungous cultures.

The Eh of three-salt synthetic culture media (modifications of Richards' solution) with a carbon source of glucose or sucrose was about 0.6 volt. The initial potential of a buffered prune-extract medium was about 0.4 volt.

The data show that the fungi employed had a definite effect on the Eh of the media in which they were grown. The oxidation-reduction potentials of the media were lowered in all cases by the various fungi during the initial period of vegetative growth.

Aspergillus niger proved to be most uniform in its effects on the media with regard to the intensity of reduction of the Eh. With the media used, the minimum Eh level established by this organism was always about 0.2 volt. The other fungi, while effecting reduction, did not produce the same Eh intensities on the various media.

The cultures of *P. granulatatum* produced the greatest reduction of Eh levels of the media in most cases. In one synthetic medium the potential was reduced from about 0.600 volt to -0.024 volt in 284 hours.

The strains of *S. fructicola* gave good growth on the buffered prune-extract medium but reduced the Eh only from 0.4 volt to about 0.3 volt. Attempts

to grow these fungi on synthetic media were unsuccessful.

Botrytis Allii produced similar effects on the oxidation-reduction potentials of the media as described for *A. niger*, except that in most cases there was a time lag before reduction began.

The intensity of reducing conditions established in the media by *P. expansum* varied from Eh 0.2 volt in the prune-extract medium to Eh 0.046 volt in a synthetic medium.

A difference in Eh of 0.2 volt in the media did not appear to have any effect on the ability of these fungi to initiate growth.

Spore production did not appear to be related to the Eh level of the media.

In the sterile media, the Eh/pH relationship varied from 55 millivolts to 38 millivolts change in Eh for each pH unit in a range of hydrogen-ion concentration from pH 2.5 to 6.0. The Eh changes produced by the fungi in the culture media did not appear to have any direct relationship with pH changes.

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CONE FORMATION IN SEQUOIA GIGANTEA. I. THE RELATION OF STEM SIZE AND TISSUE DEVELOPMENT TO CONE FORMATION.

II. THE HISTORY OF THE SEED CONE¹

J. T. Buchholz

THE VEGETATIVE stem tips of *Sequoia gigantea*, described in brief form elsewhere (Buchholz, 1937), show a remarkable range of variation in size, both in the lengths of the annual shoots and in their diameters. The diameter of the largest stem tips may exceed 4.5 mm. at 1 cm. from the tip, while that of the smallest twigs is about 1.3 mm. These larger stem-tips may be classified as leaders and sub-leaders (or leaders of side branches), according to their position and dimensions. The branching system is monopodial throughout, with a large central leader surrounded by smaller branches similar to leaders at

The leader and its side branches in a young *Sequoia* give the tree a graceful conical form, while the old trees, which have long ago attained their height, have lost the central leader and have irregular tops. Young trees, therefore, appear to have a different growth form and do not resemble the parent patriarchs of the forest which have stood for more than a thousand years. However, in both, the form of branching at the stem tip is monopodial.

Grouped among the vegetative leaders, certain twigs may bear the flowers of seed cones; others on more slender twigs may bear pollen cones. Both kinds of cone flowers are shown in figure 2. Both the seed cones and pollen cones are strictly terminal to the twigs which bear them, but cones are never formed on the ends of leaders. It was during a search for the flowers of seed cones that the writer became interested in the details of vegetative growth at the ends of branches of *Sequoia gigantea*. It happened that a severe storm following the formation of a burden of snow and ice in the region of General Grant Park brought down many large branches of the big trees. Though this happened in March, 1936, these branches were preserved in the snow at the base of the trees and could still be obtained fresh and green as the snow disappeared in April. On these branches the very small seed and pollen cones (which had formed during the previous season) could be collected for study. Also, there were many mature seed cones from previous years from which the history of their development could be determined. Each annual section of shoots in the vegetative branching system could be identified, so that dates for the years of their growth, going back six or eight years, could be determined. Successive shoot-growths for the years 1932-1935 are included in figure 2. It was largely the cone-bearing tips of branches from the old trees in General Grant Park that contributed the material of the twigs and leaders for this investigation.

I. THE RELATION OF STEM SIZE AND TISSUE DEVELOPMENT TO CONE FORMATION.—A preliminary examination demonstrated that seed-cone flowers originate from larger twigs near the tips of branches, but never from the leaders. The stout subordinate twigs next in order to the leaders usually do not bear seed-cone flowers, unless in a year subsequent to their branching. The leaders of branches of old trees may be more than 4 mm. in diameter, but the twigs bearing seed-cone flowers are usually 2.9 mm. in diameter while fresh and shrink in drying to about 2.5 mm. on herbarium specimens. Pollen cones are formed on twigs only slightly larger than the foliage twigs. Their diameter is about 1.5 mm. There are always

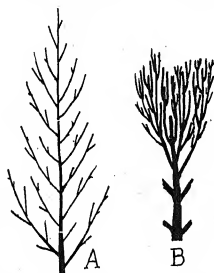


Fig. 1. Diagrams showing A, the monopodial branching at the tip of a young tree, and B, the bunched branching, also monopodial, at the tips of branches of old trees.

the tip of every branch. In young trees the central leaders are the longest and stoutest annual increments (fig. 1A), while in very old trees there is no central leader. The leaders of branches are the only ones that remain, and we find that while the method of branching is still monopodial, the leaders are often the shortest segments among the annual growth increments. This gives the twigs at the tip of a branch a bunched appearance (fig. 1B). The form of the branch, tipped by its leader, is not conical but often the reverse, having a funnel-form in which the leader is the shortest twig at the center among the annual additions of new shoots. However, the leader is always the stoutest shoot so that the axial position and larger diameter remain as the most important pair of characteristics which distinguish a leader externally from the longer side branches. Figure 2 shows the tip of such a branch with a central leader, side branches, and reproductive branches bearing cones.

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many other branches in similar positions to these flowering twigs, in fact the vegetative twigs offer a continuous series, ranging in size from the large leaders down to the smallest foliage twigs, about 1.3 mm. in diameter.

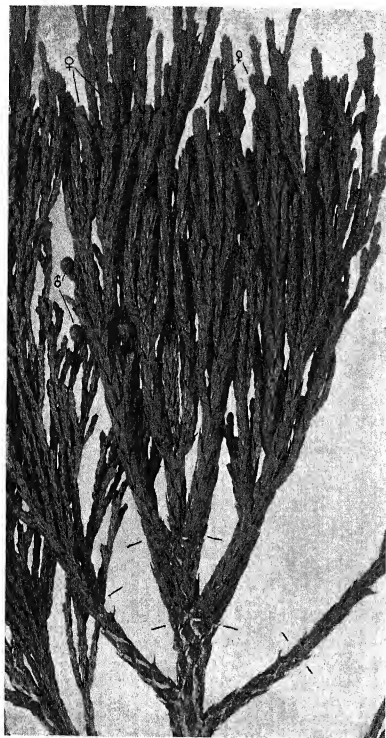


Fig. 2. Tip of branch from old tree showing the relatively short but stout leader, the longer side branches, and twigs of the second order of branching, several of which bear unopened female cones. Several small twigs bearing pollen cones are also shown. $\times 75$.

Among young trees, less than 60 years old, there were many tops which had been broken off or bent over within reach. These tips were examined and compared with the vegetative parts of the older trees. At this stage of their growth the leaders may be very long. One leader was collected which showed more than 50 cm. growth in its previous year, and several were observed with growth shoots more than 40 cm.

long. However, it was soon apparent that these young trees seldom produce seed cones. Only an occasional matured seed cone of a previous year could be observed on small trees, and since no seed-cone flowers could be obtained, the study of the leaders and side branches of these young trees was not carried beyond the preliminary stages.

After the wide range in diameter for vegetative twigs and the more restricted ranges of the twigs bearing female and male cones had been ascertained, it seemed desirable to examine the internal structure of the leaders and smaller twigs of different sizes from the old trees.

In order to answer the question as to whether there are quantitative differences among the tissues of stems of different dimensions and those which formed pollen cones and seed cones during the previous year, nearly two dozen of the tips collected from the bunched branches of old trees in General Grant Park were available. Sets of samples from about fifteen choice specimens which bore either seed cone flowers or the pollen cones, were sectioned with the microtome and stained. The samples used had been dried and all of them had leaders, side branches near the leaders, and either twigs bearing seed cones or the smaller ones bearing pollen cones. Most of the samples included some of the smallest twigs. As far as possible, series of samples were selected from the same branch. The sections were cut about midway down the length of these shoots of the previous year's growth. No growth for the season of 1936 had occurred. In length, the leaders ranged from 1.5 cm. to 3 cm. and in width from 4.2 mm. down to 2 mm. The twigs next to the leaders and the twigs bearing female flower-cones were usually longer, as explained above. The length of the smallest twigs was variable, since these were usually included in twig systems that had branched many times. The sections of the smallest twigs were taken about 1 cm. from the tips.

The transverse sections were carefully outlined (from 7-10 sections of each twig) under camera lucida, using various magnifications. Magnifications were selected so that the sets of sketches of various sizes were as nearly the same area as possible in the drawings, in order to equalize the effect of camera distortion of the edge vs. the central portion of the figures. The outline drawings showed only the total area of the section, the area of the stele (including pith), the area of the wood cylinder plus pith, and the area of the pith alone, but these were delineated as accurately as possible. Each sketch was measured with a planimeter for total area and the area occupied by these component parts, and then the 7-10 sections of each sample were averaged. Since the mm. scale of magnification from a stage micrometer was projected and included in each set of sketches, it was possible to compute the diameter as obtained from the formula $D = (\text{Area} / .7854)^{1/2}$ and convert all measurements to the mm. scale of the metric system.

The entire series of measurements from the sketches need not be given here. Table 1 presents a summary

TABLE 1. Cross-sectional dimensions of stem tips in *Sequoia gigantea* (winter conditions) with corrections made to correspond to sizes of herbarium specimens.

		Total stem			Stele			Wood cylinder			Pith	
		Diam. mm.	Area sq. mm.		Diam. mm.	Area sq. mm.	Per- centage area	Diam. mm.	Area sq. mm.	Per- centage area	Diam. mm.	Area sq. mm.
A	52 sections from 7 largest leaders	Mean 3.59 σ .35	10.23		1.27 .15	1.29 .15	12.6 2.0	1.00 .12	.800 .12	7.8 1.5	.72 .12	.410 .8
B	72 sections from 9 large leaders and near branches	Mean 3.05 σ .06	7.30		.93 .09	.686 .09	9.4 1.7	.72 .11	.395 .11	5.4 1.5	.52 .05	.213 .8
C	48 sections from 6 small leaders and side branches	Mean 2.80 σ .13	6.15		.84 .09	.555 .09	9.0 2.4	.62 .06	.312 .06	5.1 1.2	.47 .06	.173 .9
D	51 sections from 6 side branches	Mean 2.29 σ .17	4.12		.60 .08	.283 .08	6.9 1.1	.46 .06	.169 .06	4.1 1.0	.33 .05	.086 .4
E	58 sections from 8 twigs bearing female cones	Mean 2.54 σ .25	5.08		.78 .11	.475 .11	9.3 1.3	.55 .08	.244 .08	4.8 .9	.31 .05	.081 .4
F	60 sections from 7 similar side branches or small leaders	Mean 2.50 σ .30	4.90		.63 .08	.312 .08	6.4 1.0	.49 .07	.186 .07	3.8 .5	.34 .07	.090 .6
G	62 sections from 9 twigs bearing pollen cones	Mean 1.46 σ .14	1.69		.30 .02	.071 .02	4.2 .5	.20 .02	.032 .02	1.9 .2	.06 .02	.0028 .04
H	94 sections from 13 small twigs	Mean 1.40 σ .18	1.54		.27 .03	.0585 .03	3.8 .8	.18 .02	.0262 .02	1.7 .3	.07 .02	.0036 .04

TABLE 2. Method of summary, Group A of table 1.

Stems from Group A												
Means of 7 sections	4.22	13.98	1.52	1.81	13.0	1.18	1.11	7.9	.81	.52	3.7	
Means of 8 sections	3.78	11.22	1.50	1.77	15.8	1.21	1.15	10.2	.81	.52	4.6	
Means of 8 sections	3.70	10.75	1.14	1.02	9.5	.83	.61	5.7	.69	.38	3.5	
Means of 8 sections	3.64	10.42	1.25	1.24	11.9	.97	.74	7.1	.94	.32	3.1	
Means of 5 sections	3.44	9.31	1.13	1.01	10.7	.96	.72	7.7	.80	.50	5.4	
Means of 7 sections	3.21	8.08	1.21	1.16	14.3	.93	.68	8.4	.63	.31	3.8	
Means of 9 sections	3.16	7.85	1.14	1.02	13.0	.87	.60	7.6	.65	.33	4.2	
Total	25.15	71.61	8.89	9.03	88.2	7.00	5.61	54.6	5.03	2.88	28.3	
Mean, 52 sections	3.59	10.23	1.27	1.29	12.6	1.00	.800	7.8	.72	.410	4.0	

showing the vegetative and reproductive stems divided into size classes—groups A–H. In table 2 enough of the details of summary of data from Group A, including the 7 largest stems, is given so that the reader may understand the method of treatment in tabulating the quantitative data.

The percentages of stellar tissues are based on the total stem area and include the pith as part of the stele. Whether or not the pith is included with the stele would make no difference in the diameter of this tissue or in that of the wood cylinder. These data include corrections for shrinkage in the microtechnique to bring the sections back to the dimensions previously observed in the dry herbarium specimens. Allowance must still be made for shrinkage where living material is examined. The averaged values for areas were used in computing the diameters in terms of sections of perfect cylinders. The columns in the

The quantitative differences in the steles and stellar components of these 28 stems show up best if they are divided into several groups for purposes of comparison. My first impulse was to divide them into four equal groups consisting of seven stems each, but it was found that if this is done there are several stems of equal or nearly equal dimensions falling on the dividing line between groups. They do not constitute an even series of steps in their dimensions. For this reason the series of stems was divided into slightly unequal groups of 7, 9, 6, and 6 stems each, a method of grouping which would take advantage of the dimensional gaps present in the series. These four groups range in size as follows: Group A, those which were between 4.22 mm. and 3.16 mm. in diameter with a mean diameter of 3.59 mm.; Group B between 3.11 mm. and 2.95 mm. with a mean diameter of 3.05 mm.; Group C between 2.92 and

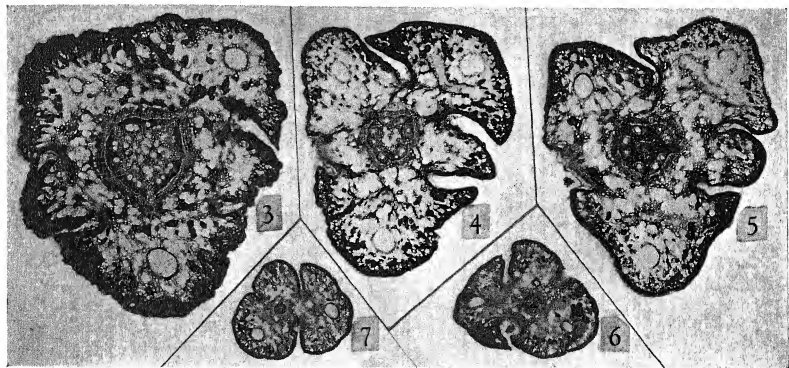


Fig. 3-7. Cross section of stems of various dimensions.—Fig. 3 from Group A.—Fig. 4 from Group D and F.—Fig. 5 from Group E.—Fig. 6 from Group G.—Fig. 7 from Group H. $\times 17$.

two tables show the mean computed diameters in mm., but each value given is the mean of 6 or more different stems averaged from 7–10 sketches, with the variability between stem samples indicated by the standard deviations (σ).

A preliminary analysis of the data when the group of leaders was compared to the corresponding group of nearest side branches showed that there was very little difference between them except the characteristics associated with other stems of comparable dimensions. They formed a continuous intergrading series.

When the 28 twigs of groups A–D were tested for the correlation between the cross-sectional area of the stem and the percentage of area included in the stele, the coefficient of correlation (r) was found to be $+ .60$ ($\sigma = .12$), and therefore significant. The stouter the stem leader, the larger the percentage of its area included in the stele.

2.62 mm. with a mean diameter of 2.80 mm.; Group D between 2.5 and 2.0 mm. with a mean diameter of 2.32 mm. It should be noted that these diameters were all reduced to the dimensions of perfect cylinders; actually they are not perfectly circular in outline, the perimeter and pith offering the widest divergence, as will be seen by consulting figure 3–5.

The female reproductive stems, discussed below, constitute another group (E), and a special group of smaller vegetative stems, selected for comparison with E was tabulated as Group F. Group G are male reproductive stems, and Group H, the smallest twigs.

Figure 3 is one of the stems of Group A; figure 4 is a section from Group D, and this stem was also included among those selected for Group F. Figure 5 is a section of a female twig of Group E.

Leaf bases were included in the cross-sectional areas, and while they offered considerable variation, the use of the means obtained from 7–10 sections tended to

equalize these variables. The variations between the stems taken at random from branches of different trees offered wider variations than different sections from the same twigs.

Group A, from 52 sections representing the 7 largest leaders, had 12.6 per cent of their areas included in the stele, 7.8 per cent of the areas were included in the wood cylinder, and 4 per cent in pith. Figure 3 comes very close to these mean dimensions. The next group, B, consisting of 72 sections cut from 9 leaders, whose mean diameter was 3.05 mm., had 9.4 per cent of the cross-sectional area included in the stele, 5.4 per cent in the wood cylinders, and 2.9 per cent in the pith. In the next two groups the corresponding proportions drop rapidly, Group D, representing the means from 51 sections of six twigs, having only 6.9 per cent stele, 4.1 per cent wood cylinder, and 2.1 per cent pith.

The group of twigs bearing small seed cones nearly ready for pollination, shown in figure 2 at the female sign and tabulated in Group E, resemble Group D most closely, the smallest of the stems considered as leaders or nearest side branches. They digress most widely quantitatively from the largest leaders shown in Group A. The mean diameter of twigs bearing female cones was 2.54 mm.; their steles were .78 mm. in diameter, and the cross-sectional area of the stele was 9.3 per cent of the total area of the twigs. The wood cylinders .56 mm. in diameter were 4.8 per cent of the cross-sectional area, and their piths with a diameter of .32 mm. were 1.6 per cent of the cross-sectional areas. Figure 5, a little above the means in dimensions, is fairly representative of Group E, the sample to which this section belongs having 9.3 per cent of its area stele, 5.3 per cent wood cylinder, and 1.9 per cent pith.

In Group F we have selected 7 of these smaller twigs (not leaders) whose diameters ranged between 2.1 and 2.93 mm. and whose pith is the smallest found in the C and D groups with areas between 1.5 to 2.5 per cent of the total cross section. Figure 4 was included in this group, but its dimensions are slightly below the mean. The mean diameter of Group F was 2.50 mm., compared to 2.54 mm. in Group E, and the area of the pith was 1.8 per cent compared to 1.6 per cent in Group E. The pith of Group F is still too large, but it is near enough to the pith in Group E to fall within the standard error. However, when we compare the percentage of stelar area in Group E with that of Group F, we find a difference of 2.9. The corresponding standard errors are .46 for Group E and .35 for Group F. The standard error of the difference $(.46^2 + .35^2)^{1/2} = .578$. The difference, 2.9, is 5 times the standard error of the difference and therefore statistically significant.

Accordingly, we may conclude that the twigs which bear the flowers of seed-cones at their tips have a larger proportion of stelar diameters and a smaller pith than other twigs with corresponding external diameters.

There are many twigs of intermediate dimensions smaller than the above and larger than the branches bearing pollen cones. Some of these twigs branch profusely and may become several times as long as the leaders. The ultimate divisions become the smallest twigs. None of these intermediate branches were included in this study.

The pollen cones are borne on twigs of a much smaller size. Nine such twigs obtained from different branches were sectioned. Figure 6 shows one of them. They ranged in diameter from 1.66 mm. down to 1.24 mm. These data were treated in the same way as the data for the larger stems and are shown in Group G. Their mean is 1.46 mm.; their steles, .30 mm. in diameter, occupied only 4.2 per cent of the total cross-sectional areas; the wood cylinder, which is .20 mm. in diameter, included 1.9 per cent of this area; the pith, .06 mm. in mean diameter, represents slightly less than .2 per cent of the area.

There is in these pollen-cone branches a distinct drop in the proportionate dimensions of all vascular components, the pith showing the greatest divergence. The area of the pith is now about one-tenth proportionately of the area of the pith in the stems of Group F, or D, and an eighth of that of the seed cone twigs, E.

The difference in the proportions of stelar area to the total area in seed cone twigs and pollen cone twigs is 9.3 minus 4.2, or 5.1. This difference is significant. The standard error of this difference is .49, so that this difference of 5.1 divided by the standard error of the difference gives us 9.6, indicating statistically, a high degree of significance in this difference between twigs bearing female and male cones.

Likewise there are differences in the area of the wood cylinder and pith between pollen cone twigs and seed cone twigs. The two groups appear to have different formulas of differentiation from their respective growing points.

Small stems representing the smallest leafy twigs are very similar to the twigs which bear pollen cones. Thirteen samples were sectioned and are shown in Group H. Their diameters ranged from 1.6 mm. down to 1.2 mm., and the mean diameter of the group is 1.4 mm. There is on the whole a greater variability in this group, but none of the values of the stelar components seem to differ as conspicuously from the corresponding values in Group G as was found for the female reproductive branches. Figure 7 shows a section of one of the twigs of this group.

It was found that many of the smallest twigs and those bearing pollen cones had actually been sampled below the last year's growth. Their wood included a narrow second growth ring. This fact would tend to increase rather than decrease the stelar area, but the amount of this increase is not great and may be negligible for our purpose here.

From the above comparisons, we must conclude that while the twigs which bear seed cones resemble somewhat the larger stems which are side branches not far removed from leaders, the twigs which bear

pollen cones differ very greatly. The latter resemble very closely the twigs which constitute the smallest branches. Both the female and male branches differ from other branches of similar external diameters in having significantly larger stelar areas and smaller pith areas.

In seeking for a standard or norm of stelar components in stems of different dimensions for comparison, we find very little in the literature which may be helpful. Sinnott (1936) has given us a series of measurements of diameters of steles and piths for a fern and of total cross sectional area and stele for a

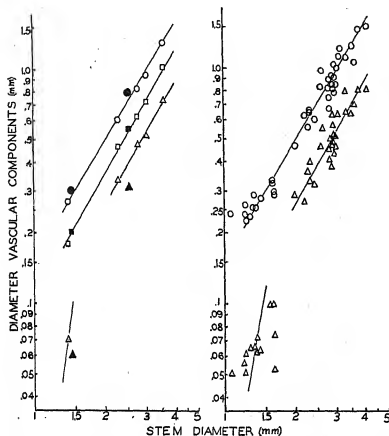


Fig. 8. Diameters of stelar components plotted logarithmically against stem diameters. In the left diagram the mean diameters of stele, wood cylinder, and pith from table 1 are plotted, with reproductive twigs shown in solid black; in the right diagram only the stele and pith of individual vegetative stems are plotted. The constant of relative growth (k) is very nearly 1.7 in all cases except for the pith of the smallest stems.

pine. Here he plotted the logarithmic values of one diameter against those of another and obtained a graph showing a straight line relationship. Figure 8 shows a similar plot for these *Sequoia* stems. In the diagram at the left in figure 8 the means from the data in table 1 are plotted; in the diagram at the right the diameters of the stele and pith are plotted individually for the 40 vegetative stems. All mean diameters are plotted logarithmically in relation to the total mean diameters of the stems of which they form a part. This type of diagram has great value in showing that in *Sequoia* stems as well as in pine stems and fern stems, these values follow this straight line relationship throughout their range of size and

that for the total diameter of the stele, even the smallest twigs fall into this relationship with the values for the steles of the largest stems. They are therefore all consistent. Likewise the diameters of the wood cylinders are found to be in fairly close agreement between the vegetative stems of all sizes. However, the mean of the pith of the smallest twigs is far out of line; it is much smaller than would be expected, a fact which was pointed out from the previous analysis.

Both the female twigs and male twigs are also plotted in solid black in the left diagram of figure 8, and these values show a greater spread. In both kinds of reproductive twigs the stelar diameters are higher than expected, while the pith is distinctly lower.

The rather wide range of differences in primary tissues found in stem tips of different kinds suggested that there might be differences already present in the dimensions and organization of the growing points, the promeristem of these stem tips. A number of the larger stem tips were examined from median, longitudinal sections and likewise a number of the smallest twigs from similar sections. Space will not permit the inclusion of illustrations, and larger numbers should be analyzed before final conclusions may be reached. However, there are important differences which are obvious in any preliminary examination. The large stem tips have not only much broader promeristem points, with many more cells, than the smallest twigs, but they seem to have promeristem masses that are not so high in relation to their width. The differentiation of tissues takes place much nearer to the promeristem in the small twigs than in large stems.

The meristems of leaders of several vigorous young trees were also examined. These have promeristems considerably broader than the leaders of the branches of old trees whose diameters are comparable, and likewise the diameters of the promeristem of small twigs of young trees were considerably broader than those of small twigs of old trees. As in the comparisons of the large and small twigs of old trees, the promeristems of vigorous leaders in the young trees are not so high in relation to their width as those at the tips of the smallest twigs.

A statistical analysis of dimensions and other characteristics of these growing points would be of considerable interest, along with the data given on the proportions of primary stelar structures found in stem tips of various sizes. After careful consideration of the factors involved, the writer feels that although such an investigation would be very desirable, it should, if possible, be supplemented by a study of leaders and other twigs collected not only between growing seasons, as in March or April, but also on different dates through the summer. Such material could be obtained in considerable abundance from young trees, but from the branches of old trees it would be very difficult to obtain the samples when needed.

One may feel assured at least indirectly from the measurements given in this paper that the promeristems of growing points differ on twigs of different sizes. One may infer something concerning the manner in which they differ because the proportions of the immediate products, the primary tissues differentiated from the promeristems, differ. The meristems of leaders and large stems produce proportionally larger steles, larger wood cylinders, and larger pith cylinders than small twigs.

This is essentially the same conclusion as that of Sinnott (1936) with respect to the relative size of the pith versus the stele in a fern and the pith versus the shoot diameter in a pine. While Sinnott states that the pith diameter increases 1.8 times as fast as the whole stem, the cortex a little more than half as fast, and the vascular cylinder at about the same rate (except in the largest stems), the writer finds that in large vegetative stems of *Sequoia* the diameter of all three tissues increases at nearly the same rate, about 1.7 times as fast as the total diameter of the shoot. The slope of the constant of relative size is different for the pith of the smallest twigs, but for the total diameter of the stele and the diameter of the wood cylinder, a value close to $K = 1.7$ seems to hold throughout the range of *Sequoia* stems.

A preliminary examination was also made of the proportions of stele, wood cylinder, and pith in the leaders and nearest side branches of young trees. Only a few of them were sampled. While the conditions were somewhat similar to those of the leaders and side branches of old trees, it was evident that the constant of relative size represented by the slope in figure 8 may differ slightly in the rapidly growing leaders. It is probably not permissible to compare these leaders of young trees which may grow more than 40 cm. per year in length with the leaders of the old trees that add only a few cm. per year without making some kind of allowance for this increased length of the shoots. Furthermore, the leaders of young trees develop considerable cambial activity which adds wide zones of secondary tissue to the stele during the first year. The material would not be comparable to the old trees unless one could allow for this in selecting the samples. Seed cones are very rare and difficult to find on young trees. One could not hope to recognize the reproductive stem tips before the cones begin to enlarge, so that a comparison between the vegetative and reproductive twigs could not easily be made.

The idea that size is an important factor in stelar morphology was first suggested and later elaborated by Bower (1930). While Bower points out great differences in the dimensions of stelar structures through a wide range of diameters and emphasizes the importance of size as a factor in stelar morphology, up to the present time there are very few investigators who have treated such material biometrically.

In the material selected by Sinnott, little or no cambial growth had taken place, and in Bower's studies with ferns there is usually no cambium, and

practically all tissues are those resulting from the differentiation of primary meristems. Such differences are due, without doubt, to differences in the growing point, the promeristem.

While all the *Sequoia* stem tips include a little cambial growth, the total cambial activity during the first year is not great in these twigs of old trees. The differences found are likewise due very largely to differences in the primary tissues differentiated from the promeristem. Since the chief difference recognizable in the promeristem from direct examination are very largely those of size, number of cells, and shape, we must resort to the quantitative differences found in the primary tissues a short distance back, or the immediate product of the promeristem, in describing the differences in these growing points. The data presented in table 1 enables us to do this.

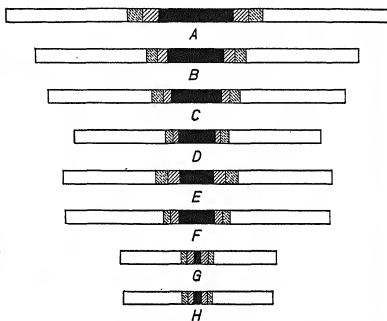


Fig. 9. Diagrams showing total relative cross-sectional dimensions and proportions of stele, xylem, and pith differentiated from the pro-meristems at the tips of stems of various dimensions. $\times 21.4$.

A large stem tip from Group A has a promeristem which gives rise to the condition shown in A, figure 9. The other groups, B-H, follow in order, with the percentage of stelar tissue decreasing, so that the formula of differentiation from the promeristem for the smallest twigs is shown in H. These diagrams may therefore be taken to represent the formulas for quantitative differences in primary tissues produced in the meristem, and the fact that the total xylem and phloem present represent only a small proportion of the whole shows that the secondary growth due to cambial activity during the first year cannot be responsible for more than a very small part of the differences.

In this series of diagrams as well as in the graph in figure 8, the female twigs E digress very distinctly from the position which they would occupy on the basis of their external dimensions. It follows that the primary meristem of a reproductive twig differs qualitatively from that of a vegetative twig, to the

extent that E in figure 9 differs from F, and G from H. In both cases the piths of reproductive twigs are smaller than the vegetative twigs of the same diameter, and there is more xylem and phloem so that the steles are larger.

II. THE HISTORY OF THE SEED CONE.—The primordia of the seed cone (female flower buds) are formed on the ends of the twigs which bear them (Group E of table 1) during the summer of a given season. As shown in figure 2, they remain small, enclosed within the terminal bud, and are scarcely noticeable throughout the winter until these flower buds begin to swell and open in the following spring. They are marked by the fine aristate tips of the cone scales which are brown in color and protrude slightly at the tips of these twigs. By the time pollination takes place, the tiny bristly cones are exposed and are still at this stage expanded to no more than two or three times the diameter of the twigs which bear them. The twigs which bear female flowers subsequently become the peduncles of the seed cone.

Pollination occurs in the latter half of April or early in May. There may possibly be differences in the time of pollination in different seasons; the only observation made by the writer was for 1936 in the region of General Grant Park. Late in June the female cones have enlarged very little, but by the middle of July they are nearly half grown and appear to be full grown by the end of the first week in August. During their period of rapid enlargement, the cones remain succulent, but during August, after they have become full grown, they rapidly become woody.

Fertilization takes place during the second week in August (1936), and the embryos of one or two cells are found developing on the ends of very long suspensor cells during September and well into October. By the time winter sets in, the embryos are still composed of only a few cells, and they pass through the winter in this condition. This is the second winter in the history of the cone. The embryos are still without cotyledons in the following June, forming the seed leaves only late in July or early in August of the second year. However, the embryos grow more rapidly now, and the seeds are found to be morphologically mature at the close of the growing season; in the second year following pollination and fertilization and in the third year following the inception of the bud primordium of the seed cones.

The remarkable thing about the seed cones of *Sequoia gigantea* is the fact that the cones are not shed when the seeds are ripe. They may remain attached to the tree for many years, to be shed gradually at some later time, many of them through some accident, such as wind or snow, the agency of squirrels, etc. Throughout this period the seeds may be retained. As soon as the cones become detached, they dry out, and the seeds are liberated within a few days. Whether all seeds are retained is a question; it seems likely that during the late summer, which is the dry season of the year when the cone scales may

shrink, some seeds may drop out from between the scales without detachment of the cones. However, it is also certain that in several cones which were more than twenty years old, a good quantity of seeds were still obtainable. From one determined definitely to be at least 19 years old, 137 seeds were counted. There is considerable variation from year to year in the yield of seeds per cone so that comparisons of the yield of single cones mean little. Six cones ranging in age from 12 to 19 years yielded an average of 164 seeds, while 10 cones from 3 to 7 years old contained an average of 167 seeds. It appears, therefore, that usually most of the seeds are retained until the cone becomes detached.

All such cones remain green, and the tissues in the cone scales remain alive. The difference between the green cones a few years old and more recent cones can sometimes scarcely be detected upon examination. Occasionally a cone which has opened, turned brown, and lost its seeds is found still attached to a tree. These dead cones are soon detached. Very old cones may be found covered with a good growth of lichens that still have chlorophyll beneath the grayed surface. Such cones may sometimes be so old that their peduncles are found inserted into stems more than 3 cm. in diameter. Since seed cones are formed only at the tips of twigs in near relation to leaders, it is obvious that these were formed when the related leader was no larger than about 4 mm. in diameter. By sawing out the disk of such a stem at the point of attachment of the cone and counting the growth rings in the wood, the age of an old cone may be determined.

However, the history of the cone is written within the peduncle itself. Figure 5 shows the peduncle of a cone shortly before pollination. Here we have the narrow growth ring of a single season's growth representing this twig in the year in which the primordium of the cone was laid down. This first growth ring shows resin canals. These usually occur in the first year's growth of the twig which becomes the cone peduncle and were present in all the larger twigs shown in our figures. Resin canals are not present in the vascular cylinder of the male branches or in the smallest twigs, and they are sometimes poorly developed in the peduncles of cones found on young rapidly growing trees.

Figure 10 shows a sector of a cone peduncle at the end of August of the year of pollination and fertilization. Here we have the narrow growth ring of the first year together with a very wide growth ring formed during the current season. Cones collected during the fall and winter which have only these two growth rings show very immature embryos in the seeds. During the following season another well-marked growth ring is produced. Cones whose seeds have just reached maturity show this condition. Such cones have three rings, a narrow growth ring, usually with resin canals next to the pith, then a very wide ring followed by another narrow ring of wood.

In subsequent seasons additional narrow rings are formed, which are closer and closer together with an occasional wider ring or a succession of wider rings interspersed. Figure 11 is a sector which shows a cone peduncle during the summer of the third season following fertilization in the cone. It has five growth rings. Figure 12 shows a sector of the peduncle of a cone in which there are a total of 16 or more growth rings.

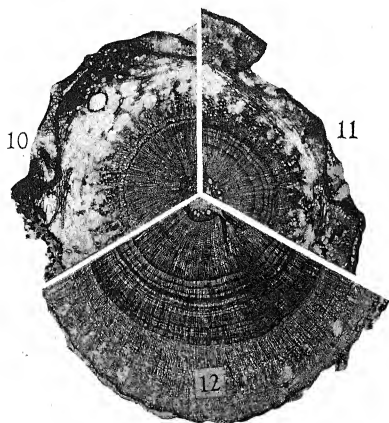


Fig. 10-12. Sectors of three cone peduncles showing, in fig. 10, the condition near the end of the year of pollination and fertilization, in fig. 11, three years after fertilization, and in fig. 12, sixteen or more years after pollination and fertilization.

Not all cones can be dated accurately. In the peduncles of some, all growth rings show distinctly; in others some of the growth rings are dropped on one side, or they may be so narrow that there is only a continuation of summer wood where one feels certain that two or more growth rings are involved and where sometimes several rings can be identified in a different radius by following a particular ring around the circumference.

There are really three methods for dating cones: (1) the growth rings in the wood of the branch to which the cone was attached (with allowance of several years for the age of the peduncle and stem below the cone before the cone was formed), (2) the growth rings in the peduncle of the cone, and (3) the growth increments of twigs shown externally by a careful examination of the branching system and the junctures between successive annual shoots. A few of these successive shoots are shown and marked in figure 2. The latter method is accurate only for cones less than a half dozen years old. While no method is entirely infallible, the combined use of all

three methods would make it possible to date the cones of *Sequoia gigantea* with a fair degree of accuracy. For cones only a few years back, the last named method would be sufficient, but for the oldest cones, the combined testimony of the growth rings in the stem into which the cone is inserted and the growth rings of the peduncle itself may make the dating of the cone reasonably certain in many instances and give at least a minimum age in others.

That the seed cones of *Sequoia gigantea* are persistent for many years is not clearly brought out in the literature of *Sequoia*. The situation is somewhat confused by the fact that in the other species of *Sequoia*, the coastal redwood, the cones mature and usually shed their seeds in the year of their pollination. Old dried cones of the previous year's crop may be found on some trees of *Sequoia sempervirens*, but these never remain living. They usually shed their seeds when ripe, are not green, and do not continue to grow in the season after pollination. Statements may be found in some taxonomic descriptions (Sargent, 1922) that the cones of *Sequoia gigantea* require two years to mature, but these statements are indefinite. It is not clear whether by the first year, the year of formation of small cone buds is meant, or whether the condition found in *Pinus* may be inferred, in which cones pollinated in one season remain small throughout that year only to begin their enlargement a year later. In pines, pollination and fertilization is separated by a little more than a year; the same is true of *Sciadopitys* and a few other genera of conifers. In *Sequoia gigantea* pollination, enlargement of the cone, and fertilization all take place in the same year, but another year is required for the maturity of the embryos.

SUMMARY

The vegetative stem tips of *Sequoia gigantea* show a wide range of dimensional variation. The diameters of stems range from leaders of more than 4 mm. down to twigs less than 1.3 mm. The larger stems have proportionately larger steles and larger wood cylinders than small stems, such that the diameters of the steles and wood cylinders increase more rapidly than the total stem diameters. This relationship may be graphically represented by the slope of a line resulting from the logarithmic plotting of the diameters of the stelar and wood cylinders against the total diameters of the stems. For the steles (including pith) and wood cylinders this slope representing the constant of relative size (k) is very nearly the same throughout the size range, but for the pith it differs in small stems.

The reproductive stems have a slightly different formula, giving larger steles and smaller pith than vegetative twigs of corresponding diameters.

These differences in stem tips are inherent in differences in the primary meristems, both for the vegetative stems of all sizes and for the reproductive stems.

The cones of *Sequoia gigantea* are formed on the ends of reproductive stems in the season previous to pollination. The female cones are borne on stems in near relation to leaders. Their mean diameter (dry) is 2.54 mm., and the male cones are borne on stems 1.46 mm. in diameter. The female cones enlarge to full size in the season of their pollination, and though fertilization occurs in August of the same season, the seeds are not mature morphologically until the end of the subsequent season. Thus at least three years are included in the history of a seed cone.

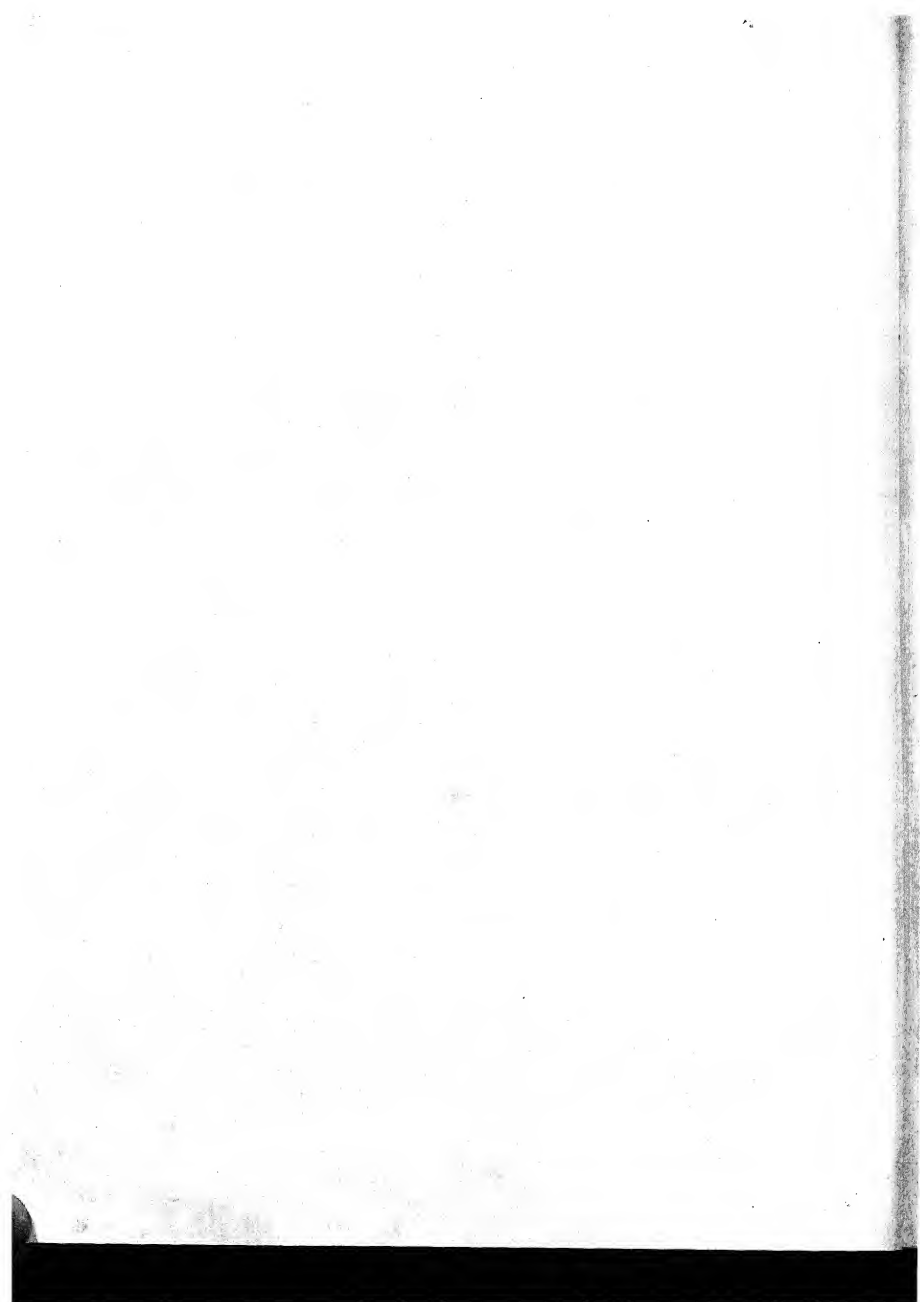
Mature cones are persistent and retain their seeds for many years after maturity of the embryos. During this period they remain green and appear to be active in photosynthesis. Their age may be determined by several methods, including the growth rings found in the peduncles of the cones themselves. Cones in excess of twenty years have been found.

This feature of retaining the seeds in the cones with continued growth of their peduncles is one which not only distinguishes the big tree from the related redwood but is a unique feature among conifers.

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A NEW FOSSIL OAK WOOD FROM IDAHO¹

Irwin Boeshore and J. Austin Jump

ALTHOUGH SEVERAL hundred species of fossil oaks from the United States have been described from their leaf impressions, comparatively little work has been done on the fossil wood of the genus. This neglect may be attributed in part to the uncertainty of specific differentiation on the basis of characters of the wood anatomy, since positive identification of oak species often cannot be made from wood structure alone. However, the sub-genera of the genus *Quercus* may be determined by the microscopic examination of their wood anatomy, and many of their included species also may be identified by this means. Thus it seems reasonable that well preserved specimens of fossil wood might be expected to show similar distinctions.

Most of the work on fossil oak wood has been done by German investigators who described a number of western American species as well as European ones. This work is so little known that Trelease (1924), in monographing the oaks of America, stated that the only fossil oak wood to receive a specific name was *Quercus Marcyana* Pen., although at that time more than a dozen species had been described. However, the descriptions of these oaks might have been overlooked very easily since they were not published in widely distributed journals.

The first description of fossil oak wood was published in 1839 by Göppert, who described fossil wood from Silesia and northern Germany and named it *Klädienia quercoides*. At that time he mentioned the nearly complete agreement of *K. quercoides* and *Quercus pedunculata* and six years later changed the generic name to *Quercites*, the genus in which fossil leaves of oak are now placed. However, Unger in 1842 had proposed the genus *Quercinum* for fossil oak wood and had pointed out that probably *Klädienia* Göpp. should be changed to *Quercinum*. Very little notice was taken of the genus until Felix (1883, 1884) published the descriptions of the species known at that time and added four new ones. In the first of these papers he showed that the three species previously described by Unger were based on invalid characters and therewith included them in the group of species upon which he published. Penhallow (1891) described a fossil wood from the post-glacial of Illinois that strongly resembled the modern chestnut oaks and named it *Quercus Marcyana*, disregarding the established generic name *Quercinum*. Knowlton (1899) published a description of a new species, *Quercinum lamarensis*, and nothing further appeared in the literature until Platen (1908) added five new species to the genus. Since that time little has been published except for two reports by American workers on fossil woods from California, one of which was apparently identical with the modern *Quercus agrifolia*. These woods were given the generic name

Quercus, although it would appear that less confusion would result if the genus *Quercinum* were adhered to when fossil wood is being described.

The specimen which is described in this paper was obtained from the Miocene Payette formation in the vicinity of Payette, Idaho. The only modern oaks of Idaho are confined to the southern part of the state and occur rather sparsely, but a number of fossil oak leaves have been collected from the state, and three species of *Quercites* have been described from the same locality in which this wood was found. The fossil oak herewith described belongs to the sub-genus *Leucobalanus*, and the name *Quercinum album* sp. nov. is proposed by the authors, since it differs from previously described species in characters enumerated in the accompanying table. The specimen was a piece of heartwood, as seen from the presence of tyloses. The microscopic structure was fairly well preserved, and the color was orange-yellow, with white lines denoting the course of the large wood rays.

Quercinum album sp. nov.—*Transverse*.—Annual rings 1.3–1.8 mm. wide. Rays conspicuous; broad rays spaced at irregular intervals and up to 500 μ wide; small rays may continue through a ring. Transition from spring to summer wood vessels very abrupt. Vessels in spring wood very large elliptical, ovate, or sub-orbicular, measuring up to 450 μ in length and 340 μ in width, plugged with conspicuous flattened tyloses. Summer wood vessels barely visible with a hand lens, thin walled, averaging 30–75 μ in diameter.

Radial.—Pits in tracheids elliptical to orbicular, 5–7 μ up to 8–12 μ ; tori sometimes oblique in reference to tracheid walls.

Tangential.—Broad rays 20–30 seriate in width; individual cells frequently hexagonal due to mutual pressure, 8–25 μ in diameter; lumen round to elliptical. Narrow rays abundant; both uniseriate and biseriate, with the uniseriate type predominating. Both types are occasionally found in the same region, but the groups are composed more often of a single type. Height of narrow rays varies from 2 to 20 cells along the grain, averaging 12 to 15, but occasionally as many as 30. The biseriate rays are usually somewhat shorter than the uniseriate.

The accompanying table contains the principal anatomical characteristics of all the species of *Quercinum* which were found in the literature. Felix (1883) mentions *Q. rossicum* Mercklin in reference to its similarity to *Q. montanum* Felix, but the authors of the present paper were unable to obtain the publication in which *Q. rossicum* was described.

The literature cited includes references to the original descriptions of all the fossil oaks referred to in this paper.

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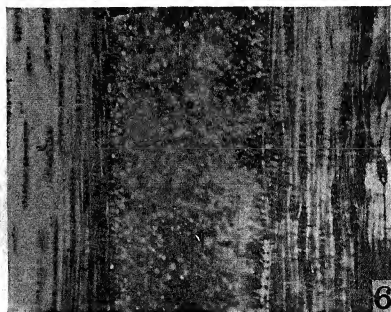
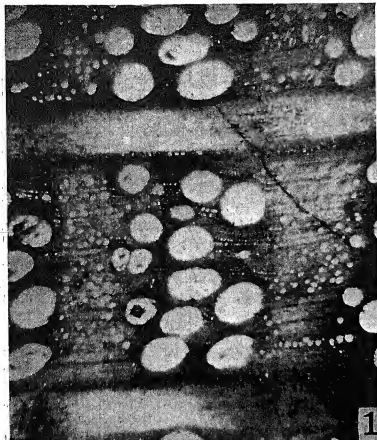


Fig. 1-6.—Fig. 1. Transverse section of *Quercinium album* showing annual rings, broad rays, and abrupt transition from spring to summer wood.—Fig. 2. Tangential section of *Q. album* showing pits in wall of a vessel.—Fig. 3. Radial section of *Q. album* showing pits in tracheid walls.—Fig. 4. Tyloses and pits in a vessel of the spring wood of *Q. album*.—Fig. 5. Wood ray of *Q. album* in radial section.—Fig. 6. Portion of broad ray and uniseriate and biseriate small rays of *Q. album*.

TABLE 1. Anatomical characters of all described species of *Quercinum* found in the literature.

Species	Spring wood	Summer wood	Rings	Small rays	Large rays	Pits	Remarks
<i>Q. Abromeiti</i> Platen	Vessels 212 μ to 350 μ ; flattened tangentially.	Vessels 50 μ to 62 μ ; not in rows.	Up to 1.5 mm. wide.		Up to 450 μ wide; 1.5 mm. apart.	7.5 μ in diameter.	A root wood.
<i>Q. agrifolia</i>							Identical with the modern <i>Quercus agrifolia</i> .
<i>Q. anomalum</i> Platen	Vessels 338 μ radially and 300 μ tangentially.	Vessels 87 μ radially, 75 μ tangentially.		Up to 30 cells high. Lumen of cells 12.5 μ .	1 to 1.5 mm. apart.	Simple 17.5 μ wide. In ray parenchyma.	
<i>Q. Bäckhianum</i> Felix	Vessels 220 μ rad. and 290 μ tan., or round and up to 320 μ in diameter.	Vessels circular, 30 μ to 50 μ . Also a few 10 μ wide among others.					Otherwise resembling <i>Q. compactum</i> .
<i>Q. compactum</i> Schlei.	Vessels 360 μ rad. 461 μ tan. Often with tyloses.	Vessels 80 μ by 120 μ .		Uniseriate.			Similar to the modern <i>Quercus lusitanicum</i> Lam.
<i>Q. helictozylodes</i> Felix	Vessels round, up to 300 μ . Averaging 150 μ .				2.5 mm. apart, up to 400 μ wide.		A root wood.
<i>Q. Knoultoni</i> Felix	Vessels 500 μ radially, 210 μ tangentially, tyloses present.	Vessels 80 μ by 120 μ .		Uniseriate.	1.5 mm. apart 170 μ wide.	Small, oval, bordered.	
<i>Q. lamarense</i> Knowl.	Vessels round, 160 μ to 230 μ . Not in radial rows.	Vessels 100 μ to 120 μ .		Mostly pass from one duct to the next in transverse section.	10-20 cells wide. Walls thin.		Allied to <i>Q. Knoultoni</i> , but differing in size and shape of ducts.
<i>Q. leptotricum</i> Schlei.	Vessels large, to 570 μ radially and 390 μ tangentially.			Uniseriate, up to 25 cells high.		Large; in longitudinal rows.	
<i>Q. Lesqueruzi</i> Platen	Vessels 250 μ rad. and 175 μ tang. Round tyloses.	Smallest vessels 75 μ .		Up to 30 cells high. Single cells 20-25 μ to 50 μ in dia.	Up to 175 μ in width.		
<i>Q. Marcyana</i> Pen.	No obvious distinction between spring and summer wood.	Vessels 200 μ to 300 μ . Tangentially compressed.					Incomplete description, but resembles the modern chestnut oak.

TABLE 1. Anatomical characters of all described species of *Quercinum* found in the literature. (Cont.)

Species	Spring wood	Summer wood	Rings	Small rays	Large rays	Pits	Remarks
<i>Q. montanum</i> (Marek.) Felix	Vessels large, reaching $570\ \mu$ radially, $390\ \mu$ tangentially.			Uniseriate, up to 28 cells high.	20-30 cells in width.		Similar to <i>Quercus toza</i> Bosc. and to <i>Quercinum rossicum</i> Merck.
<i>Q. primaevum</i> Göpp.	Vessels $470\ \mu$ radially, $350\ \mu$ tangentially.	Gradual transition to summer wood.			1.6 mm. apart.	$63 \times 7.6\ \mu$. Elliptical bordered pits.	Resembles the living <i>Quercus castanaefolia</i> Mey.
<i>Q. ricardensis</i> Web.	Vessels $112\ \mu$ by $77\ \mu$.	Vessels slightly smaller than the spring wood.	37 to 12 mm. wide.	Mostly uniseriate, 3-22 cells high. Cells $10-14\ \mu$ in diameter.	375-450 μ wide. Individual cells 7-25 wide.	3.5 to $5\ \mu$ in diameter.	
<i>Q. Solerederi</i> Platen	Vessels $162\ \mu$ rad., $125\ \mu$ tang.	Vessels $63\ \mu$ radially, $50\ \mu$ tangentially.	Up to 3 mm. wide.			In vessel walls, $5\ \mu$ wide.	Calcium oxalate crystals often present.
<i>Q. Staubi</i> Felix	Vessels up to $490\ \mu$ rad., $400\ \mu$ tang., in concentric rings. Tyloses present.	$90\ \mu$ rad., $80\ \mu$ tang.		5-15 cells high. 20 high occasionally.		As in <i>Q. primaevum</i> .	
<i>Q. transiens</i> Conwentz							Similar to <i>Q. Knowltoni</i> but with abrupt transition from spring to summer wood and less large rays.
<i>Q. vasculosum</i> Schlei.	Vessels in two rows, crowded; often with tyloses. $450\ \mu$ rad. $300-400\ \mu$ tang.	Abrupt transition to summer wood.		Uniseriate, up to 15 cells high.	3 mm. apart. Up to 30 cells in width.	As in <i>Q. primaevum</i> .	
<i>Q. Wardi</i> Platen	Vessels $250\ \mu$ radially, $200\ \mu$ tangentially.		Up to 8 mm. in width.			In vessel walls $10\ \mu$ wide.	An evergreen oak.
<i>Q. album</i> sp. nov.	Vessels up to $450\ \mu$ radially and $340\ \mu$ tangentially. Plugged with flattened tyloses.	Vessels thin walled, $30\ \mu$ to $75\ \mu$ in diameter.	13 to 18 mm. wide.	Uniseriate and biseriate, averaging 12 to 15, and up to 30 cells high.	20-30 cells wide. Cells 8-25 μ in diameter.	Elliptical to orbicular in tracheids; 5-7 μ up to 8-12 μ . Tori often oblique.	

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A NEW FOSSIL PLANT FROM THE REED SPRINGS FORMATION OF SOUTHWESTERN MISSOURI¹

J. E. Cribbs

IN 1934 the writer described a petrified tree trunk from the Reed Springs Limestone of southwestern Missouri. That, I believe, was the first petrified wood reported and described from this formation.

I received recently from Dr. Ralph Voris of Southwest Missouri State Teachers College part of a second trunk found near Crane, Missouri. Later, when visiting this location, the undisturbed remnant was observed in place and removed (fig. 22, Pl. 4). This trunk, like the first described, was embedded in chert of the Reed Springs Formation.

DESCRIPTION AND STATE OF PRESERVATION.—The dimensions given here are of relative significance only, since the diameter is incomplete and the original length is unknown. When found, it was about 70 cm. long, 16 cm. wide, and 8 cm. thick. Before it was embedded, the trunk had been broken transversely. After it was exposed at the edge of a stream, an unknown amount eroded from the basal end. What remained was straight, without branches, and of uniform width.

Compression at right angles to the bedding plane flattened the original cylinder and crushed the secondary wood. This distortion did not affect it uniformly but was limited to definite regions. Transverse and longitudinal fractures are numerous. In most instances these have been filled with chert.

All cortical structures and an unknown amount of secondary wood were lost before infiltration took place. Pith was present in part of the stem, between two transverse fractures (fig. 2, Pl. 1). This remnant measured about 3 cm. in the axis of length, and had a diameter of 2 cm. A longitudinal fracture affected the pith, so that approximately three-fifths of the transverse sectional area persisted (fig. 26, 28, Pl. 4). The central cylinder of pith and some of the adjacent

secondary wood were eroded from the more apical part of the stem, and the cavity so formed was filled with limestone (fig. 22, Pl. 4). Many leaf trace bundles are visible on the inner surface of the secondary wood; and in all instances, except where obscure because of extensive erosion, they could be observed occurring in pairs, one of which entered the wood at a higher level than the other (fig. 2, Pl. 1).

Without considering the unknown amount of wood that was lost by weathering, the diameter of 16 cm. indicates an arboreal habit (fig. 1).

Infiltration was by siliceous materials. Treatment with hydrofluoric acid leaves a residuum of brown or reddish matter which falls apart, retaining none of the normal structure. Chemical replacement is incomplete, especially as regards the middle lamella region of the radial walls. Because of this condition, it was not possible to prepare satisfactory transverse sections. Apparently the ease with which the cells separate in a radial plane is due in part to the cell arrangement. The radial walls are relatively flat and meet so as to form a definite plane, whereas in the tangential dimension there is more of an alternation in cell grouping. The pith, because of more complete chemical replacement, was less friable than the secondary wood.

Spherulites are abundant in the wood zone, especially a few centimeters from the pith. Weathering, presumably subsequent to surface exposure, has caused the material between the spherulites to break down, thus contributing to the friability and increasing the difficulty of sectioning. The preservation of pits, wall thickening, and inclusions is sufficiently complete to afford an accurate interpretation of the finer structural features.

THE PITH.—A transverse section of the pith shows an irregular mass of chert in the central region about the edges of which the cells are crushed and in various stages of disintegration. The marginal portion is

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The Reed Springs Formation is of Mississippian age and overlies the Kinderhook, which, in this region, rests unconformably on Devonian.

well preserved except for a few narrow compression zones where the cells are more or less crushed and obscure.

The pith is continuous and is composed of cells that vary in size and shape. Most of them are elongated radially, except toward the center where they are approximately isodiametric, and adjacent to the secondary wood where they are smaller than elsewhere and may be elongated vertically. Radial elongation is most conspicuous centripetally from the primary xylem strands, especially those which are in contact with secondary wood. The average dimensions of pith cells are: radial 215μ , tangential 185μ , and length 125μ .

There are no tracheids, canals, sclerotic nests, or other structures present to interrupt the uniformity of the pith. Certain of the cells, however, are more

or less completely filled with a dark inclusion which may represent either secretory or excretory material. These cells are normal parenchyma elements, so far as can be determined, and cannot be distinguished from the others by size, grouping, or position.

PRIMARY STRANDS.—As seen in transverse section, the pith has in it numerous primary strands, about half of which are not in contact with the secondary wood but form a ring near the edge of the medullary tissues (fig. 1). These strands are separated from the secondary wood by a distance that varies from one to ten times their own diameter (average separation 400μ). In this regard they resemble the sub-marginal strands of *Pitys antiqua* (Scott, 1902), except that in this stem more of them are in contact with the wood.

Between twenty and thirty primary strands appear in a transverse section of the pith preserved. Since approximately three-fifths of the cross-sectional area is estimated to be present, the number of strands was about forty-five. They are small. The average radial dimension is about 115μ and the tangential 172μ . Whether or not some of the sub-marginal strands connected with marginal ones at other places than nodes could not be determined, but such connections did not occur in the small amount of pith preserved.

The sub-marginal strands are smallest in the lower part of their course, where some of them were observed to arise at the nodes as reparatory strands. The number of xylem elements, as seen in transverse section of the embedded strands, varies from seven to sixteen (average 12). The protoxylem elements vary in number from two to four and, with few exceptions, lie at the inner edge of the bundle. This endarch arrangement is apparently due to elimination of centripetal xylem, since strands in the sub-marginal position are frequently crescentic or U-shaped with the concavity centripetal. A few of the sub-marginal strands are eccentrically mesarch with the protoxylem close to the inner edge.

Most marginal strands are endarch. They may be radially elliptical in the lower part of their course but become more rounded as they approach the nodal position (fig. 8, 9, Pl. 2). The metaxylem in contact with secondary wood tends to become radially arranged, and near the node a few cells develop centripetally to the protoxylem, thus producing an eccentric mesarch condition. In the marginal position, the primary strands seldom exceed 165μ in tangential diameter before they begin dividing preliminarily to entering the secondary wood.

Protoxylem elements average 13.5μ in diameter and the metaxylem about 45μ . The latter are approximately the same size as tracheids of the secondary wood.

The primary strands are not confluent as in most species of *Calamopitys* (Scott, 1913), but are distinctly separate and spaced more or less uniformly at intervals that average 1.2 mm., which is about seven times the tangential diameter of the strand.

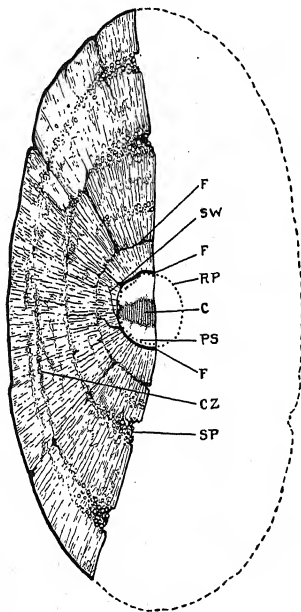


Fig. 1. A restoration, representing a transverse section of the stem. The shaded portion is all that remained where pith was found. The complete circumference shows the outline of the stem about 50 cm. above (toward the apex). C, chert; CZ, compression zones; F, fracture; PS, primary strands; RP, restoration of pith cylinder; SP, spherulites; SW, secondary wood segment that contained two pairs of emerging leaf trace bundles. $\times 4$.

Protoxylem and metaxylem elements are shown in figure 5, Plate 1, as they appear in radial section of a sub-marginal strand. Two protoxylem elements with spiral thickening are visible at the inner edge. A metaxylem element at the left appears to be centripetal but overlaps the protoxylem and is lateral to it. Metaxylem elements are scalariform, reticulate, and transitional from reticulate to pitted. They have transverse or oblique end walls. Distinctly pitted tracheids could not be observed in the sub-marginal bundles but may appear in the marginal ones before they turn out at the nodes. One or two parenchyma elements may lie among the tracheids of the strand while the latter is either marginal or sub-marginal in position. Pith cells adjacent to the embedded bundles commonly assume the appearance of a bundle sheath.

COURSE OF THE PRIMARY STRANDS.—Those primary strands which lie embedded in the pith turn out at higher levels where they come in contact with the secondary wood. After assuming the marginal position, each strand extends apically to the node directly above. There it divides, forming paired bundles which sink into the secondary wood and turn out as leaf trace strands. Since the sub-marginal bundles equal or exceed in number those in a marginal position, and arise singly at nodes as reparatory strands, they probably assume the marginal position about midway between successive nodes of the same orthostichy.

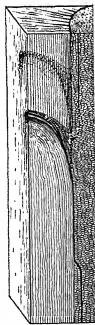


Fig. 2. A diagram reconstructed from transverse radial, and tangential sections, showing the course of primary strands, the emergence of paired traces, and the formation of a reparatory strand. In the lower bundle the primary strand has divided. A parenchyma embayment subtends each outgoing trace.

Each marginal strand divides tangentially immediately below a node. One of the pair thus formed begins at once to sink into the secondary wood. The other continues apically either to the right or left of the parenchymatous embayment above the first trace and enters the secondary wood from 1–10 mm. above

and slightly lateral to the first. A reparatory strand, which is given off into the pith from the upper bundle before it enters the wood, quickly assumes a vertical position (fig. 2).

The above description of the nodal condition is based upon the two pairs of bundles that turned out from strands in the short portion of pith preserved and upon the relative positions of paired traces as seen on the inner surface of the secondary wood (fig. 2, Pl. 1). Sections cut in an attempt to determine the nodal condition were somewhat unsatisfactory because a fracture followed the boundary between pith and wood in much of this area. At one node, a section passed through the dividing point of the bundle where a reparatory strand diverged from the inner side of the delayed or upper strand. Another section cut 1.5 mm. above that level shows a small reparatory strand buried in the pith. A section at the point of division of the second bundle shows a double arrangement of protoxylem and the initial divergence of the reparatory strand (fig. 10, Pl. 2).

In transverse sections of the pith, one strand only was observed to divide tangentially while in the sub-marginal position. It is probable, therefore, that lateral anastomosis of the embedded strands did not occur frequently. In the small amount of pith preserved, the marginal strands did not divide except at the nodes, where paired branches emerged as leaf trace bundles and a centripetal branch formed the reparatory strand. Whether or not other branching occurred could not be determined, but all evidence indicates that the marginal strands were upper extensions of those which at lower levels were embedded, for no bundles occurred in the pith opposite the marginal ones.

There was no essential difference between the two bundles of a pair. Each passed into the secondary wood gradually and, when well embedded, turned abruptly so as to lie almost but not quite at right angles to the stem axis. Secondary wood adjacent to the emerging bundles was deflected outward, mostly on the abaxial side. An elongated break or pocket occurred in the stele above each trace, into which radially elongated parenchymatous elements extended (fig. 2). Tangential and radial sections show, above this parenchyma, a crescent of short tracheids which may bear pits on all their walls.

The leaf trace bundles extend from the pith into the secondary wood about 5 mm., where they terminate and are closed over by secondary tracheids. As shown in figure 12, Plate 2, the parenchyma thins out and may end short of the bundle terminus. In other instances, the parenchyma extended to that point and apparently formed a shallow gap in the stele.

The bundles terminate in an irregular manner. Obviously no abscisus layer was formed. In each instance the secondary wood was interrupted beneath the bundle proper and was opposed by callus tissue, evidence that some secondary wood was carried out into the cortex with the traces.

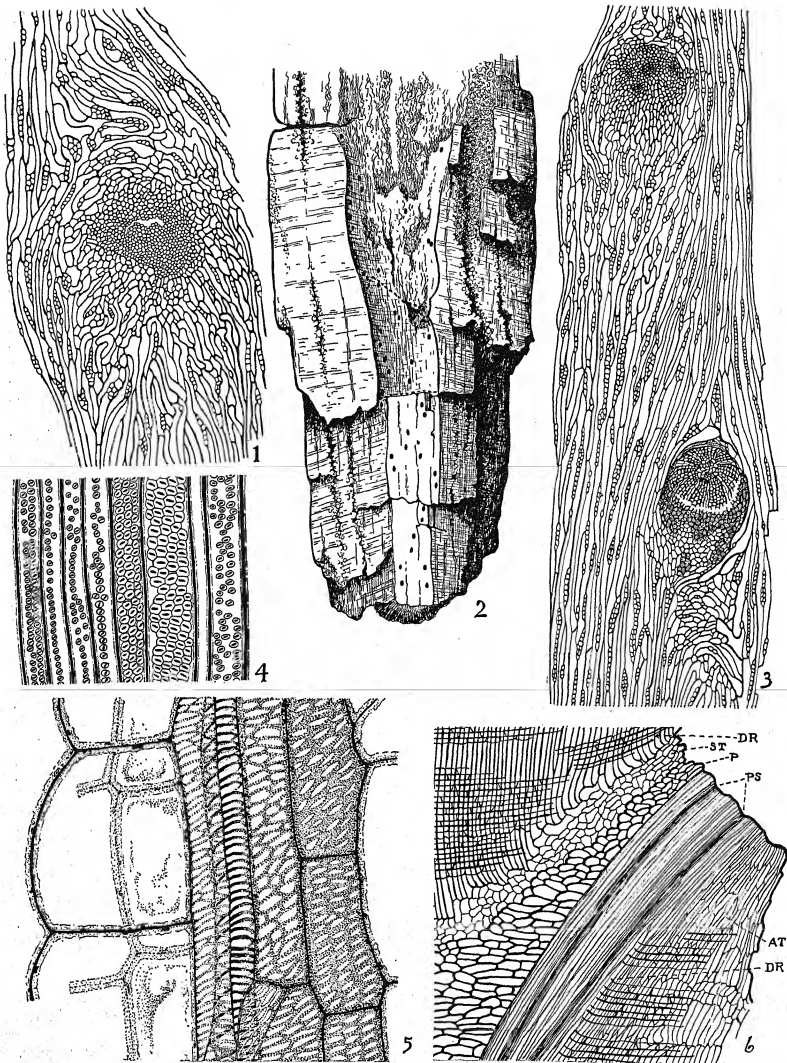


Plate 1. — Fig. 1-6. — Fig. 1. A tangential section cut about 2 mm. out from and opposite the terminus of a trace bundle, showing the distorted arrangement of tracheids and the central group of radially elongated elements. $\times 11$. — Fig. 2. Habit of a portion of the stem. The region occupied by pith is indicated by dotted lines near the center. Note the paired traces one of which lies above the other. $\times \frac{1}{2}$. — Fig. 3. A pair of traces about 5 mm. apart. The

In most sections a fairly well defined difference in the width of wood elements is visible near the terminus of the bundles. This appears to mark the end of a season's growth and the outer boundary of the secondary wood while the leaves were functional.

Radial sections of outgoing trace bundles show wood rays traversing their secondary elements on the abaxial side (fig. 6, Pl. 1). These rays are deflected slightly near the primary xylem of the bundle.

The emerging bundle, including its secondary wood, is about 1 mm. in tangential diameter near the pith and about .7 mm. distally. Secondary tracheids of the trace are narrower (average 28μ) than those of the adjoining wood and bear four or five series of crowded pits on the radial and tangential walls.

The primary strand of the emerging trace is mesarch. Well out in the wood it is surrounded by radially arranged secondary tracheids which are more extensively developed on the abaxial side. Sometimes the primary strand divided distal to the node. When this occurred, the two parts lie within the trace on a vertical plane, one near the abaxial side and the other directly below or external to the first (fig. 6, Pl. 1). Primary elements of the outgoing strand bear scalariform, spiral, and reticulate markings, and comprise an inconspicuous part of the strand as seen in transverse section.

Large callus cells meet the broken face of the strand and give way to narrow elements of mixed parenchyma and tracheids, which may continue outward 2–3 mm., maintaining the outline of the bundle, beyond which normal tracheids gradually replace them.

The general indication is that the leaves persisted through one growing season, after which they were shed and replaced by others at higher levels. Whether or not both bundles of the pair entered the petiole of a single leaf could not be determined with certainty in the absence of structures external to the secondary wood. This seems most probable, since there is no evidence that the more apical bundle entered an axial fruiting structure of the cordaitan type. There is no appreciable difference in the structure of the two. Each bundle carried out some secondary wood, terminated at the same level, and was approximately the same size. These features are the same where the traces are 1 mm. apart as where they are separated by a distance of 1 cm.

PHYLLOTAXY.—The phyllotaxy could not be determined with certainty but was evidently a complex spiral. Two features made difficult the determination of leaf arrangement. First, that portion of the stem

where outgoing traces are visible at no place represents a complete transverse sectional area; and second, multiple fracturing, together with distortion due to compression, affected to some extent the original position of the traces. At two places, the traces appear to be directly over others in the fifth spiral below. Three successive pairs in the spiral occur in approximately one third of the stem circumference. These visible spirals are obviously parastichies, and the course of the true spiral is too obscure to determine, other than it is of a complex type.

SECONDARY WOOD.—The secondary wood is dense and of the cordaitan or araucarian type. Tangential sections cut 2 cm. or more from the pith show narrow rays prevailing uniseriate or biseriate in part. Within 0.5 mm. of the pith, rays are broader, commonly 2–3-ranked or rarely 4-ranked in part. Near the pith the secondary tracheids are of normal length and width. They frequently curve about the rays and may have transverse end walls (fig. 1, Pl. 3).

Transverse sections show the tracheids are wider in the radial than in the tangential dimension. The average size is $42\mu \times 38\mu$. Obscure zones of growth occur. These are at intervals that vary from 1–5 mm. Large elements corresponding to "spring wood" form a zone 5–12 cells wide and grade outward into a wide zone composed of much smaller cells. This zonation is less clearly defined than in modern plants, yet it is sufficiently distinct to present a ringed appearance.

The radial rows of secondary tracheids meet those above and below in a suture-like line that is even and horizontal. Sometimes in regions apart from leaf traces the tracheids depart from the normal arrangement or shape and form irregular patterns or lie at an oblique angle with those of the adjoining radial series. These irregularities are not due to compression or distortion after maturity, since the wood rays traverse such areas unaffected. Other irregularities occur, such as shown in figure 11, Plate 2, where, in longitudinal series, wide tracheids are abruptly replaced by narrow ones.

There is considerable distortion of tracheids about the emerging traces, a feature which is continued beyond and opposite the terminus of a bundle. This distortion may affect the wood for a distance exceeding 1 cm. and, for 3–5 mm. outward, may retain the general outline of the bundle. These distorted tracheids give way gradually to normal ones farther out in the wood. Tangential sections, cut beyond the terminus of a bundle, present the general appearance described by Arnold (1930) and other writers. In this stem it is clearly due to the disturbance of tra-

lower is cut near the terminus, and the upper slightly off the end of the bundle. A radial arrangement of secondary elements is visible in the lower bundle. The group of tracheids immediately below the trace passed out with it. $\times 11$.—Fig. 4. A series of tracheids selected from a single radial section, showing the variation in pitting. The largest tracheid corresponds to "spring wood," where crowding of pits is typical. $\times 125$.—Fig. 5. A longitudinal-radial section through a sub-marginal strand. The scalariform tracheid at the left overlies the innermost spiral element and is lateral to it. Elements transitional between scalariform and pitted occur at the right. $\times 250$.—Fig. 6. A radial section of a trace in the secondary wood. AT, secondary wood that passes out with the trace; DR, deflected rays; P, subtending parenchyma; PS, primary strands of trace; ST, short tracheids that form a crescent over the parenchyma embayment. $\times 11$.

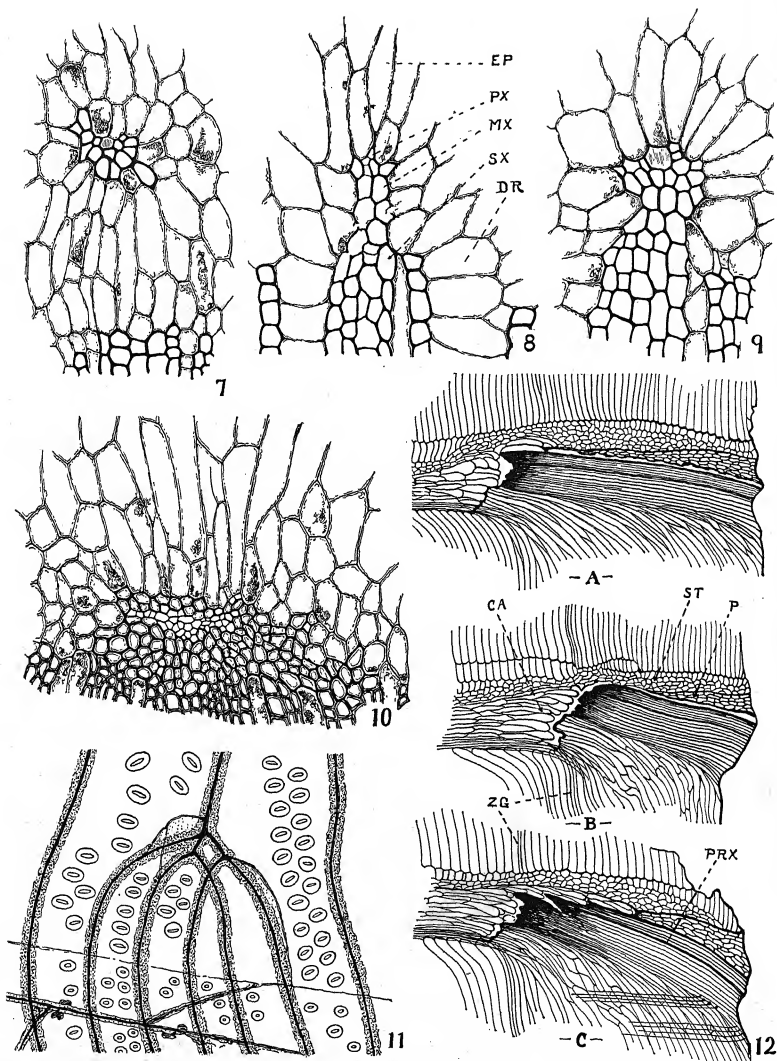


Plate 2.—Fig. 7–12.—Fig. 7. A sub-marginal strand, showing endarch arrangement, reduction of centripetal xylem, one included parenchyma element, and distinct separation from secondary wood. $\times 110$.—Fig. 8. A bundle cut at the point where the submarginal strand assumes a marginal position. *DR*, dilated ray cells; *EP*, elongated pith cells; *MX*, metaxylem; *PX*, protoxylem; *SX*, secondary xylem. $\times 110$.—Fig. 9. A marginal strand cut nearer a

cheids off the terminus of a leaf trace bundle and appears only when sections are cut external to that point. Serial tangential sections will probably confirm this interpretation for *Calixylon*, and for certain other gymnospermous wood where oval or elliptical distorted areas have been described.

WOOD RAYS.—Wood rays may be dilated where they meet the pith, due for the most part to tangentially widening their cells, although the larger rays may be four cells wide. The maximum width, where dilated, is about $250\ \mu$ (average $102\ \mu$). Rays which are wide at the pith become narrow rapidly so as to attain the uniseriate condition less than 1 mm. out in the wood.

When viewed in radial sections, ray cells near the pith are seen to be approximately square and to have thick walls. At a distance of 1 mm. from the pith, the ray cells are elongated radially and are passing into the typical muriform arrangement where they have an average length of $197\ \mu$, which is about 4.5 times the width of adjoining tracheids. Their end walls may be transverse or sloping. Granular organic remains are of frequent occurrence within the ray cells.

Rays vary from 1–49 cells in height. Most frequently they are 10–15 cells high. The individual cells average about $25\ \mu$ wide and $37\ \mu$ high. There are occasional rays composed of large cells, or more commonly these lie lateral to the ray. Among these larger elements, tracheids sometimes occur. The latter are elongated radially, but less so than the typical parenchyma cells. They may bear pits on both lateral and end walls. As observed in radial sections, these tracheid elements appear to be limited to regions of transition. They have been observed to occur under one of three conditions: First, where a new ray was intercalated between tracheids by a local change in the activity of certain cambial cells, presumably as in modern plants. In this instance, the tracheids extend radially a short distance and are then replaced by normal parenchyma. Second, tracheids may appear where rays were increased in height by the addition of marginal elements. This probably came about when cambial cells above or below an existing ray added to its elements by changing their activity (Pl. 3, fig. 16, 20). Tracheids may also appear in rays formed in the distorted secondary wood opposite the terminus of a bundle (Pl. 4, fig. 30). So far as could be determined, they appear to be limited to what might be called transitional tissues, for at no place were they observed to displace the elongated ray cells of the normal type.

Near the outgoing leaf trace bundles, rays are not so high as elsewhere and are frequently three cells wide.

PITTING.—Pits are uniformly distributed on the radial walls of the secondary wood. They are typically round or hexagonal, have a slit-like pore and are fully bordered. In the broader tracheids corresponding to "spring wood," they appear in three or four series, where they are crowded, alternately arranged, and prevalently hexagonal. As the broader elements grade outward into narrower ones, the pits become reduced to two, or rarely to one vertical series (fig. 4, Pl. 1). In the narrower elements, the pits are relatively small, are typically rounded and contiguous, or occasionally scattered. Rarely they may be irregular. In a general way, the size, crowding, and seriation varies with the width of the tracheids, but sometimes wide tracheids occur with one or two rows of pits.

In the crescentic zone of short tracheids on the adaxial side of an outgoing trace, pitting may occur on all walls. Tangential pitting frequently occurs on the distorted tracheids that accompany an outgoing trace. These pits are small and scattered (Pl. 4, fig. 19). Pits are commonly 4–5-seriate in the radially arranged secondary elements of the outgoing trace and are characteristic of both radial and tangential walls.

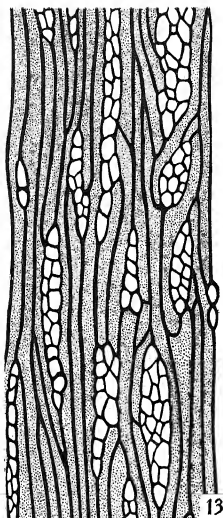
On the lateral walls of ray cells, pits are somewhat smaller than are those of the secondary tracheids and average about 8 (6–14) per tracheid for the border cells and 6 (2–12) for the inner cells.

AFFINITY.—Certain structural features of this stem indicate relationship with known cordaitan forms. In some respects it resembles the *Pityae*. In other regards there are indications of a calamopitoean affinity, especially with that line represented by *Eristophyton* and *Endoxylon*. Because of the absence of leaves and reproductive structures, its definite placement in regard to known forms is somewhat uncertain.

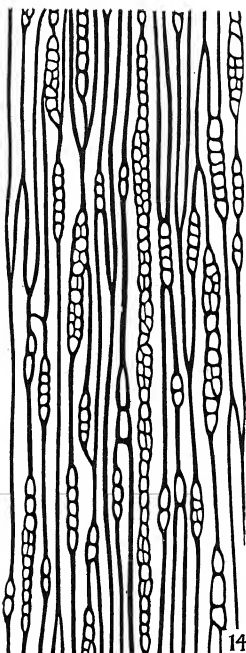
The large number of primary strands, grouped in a ring near the margin of the pith, is a feature which has been emphasized as a characteristic of the genus *Pitys* (Scott, 1902). The recent investigations of Gordon on this genus have shown that additional strands lie deeply buried in the pith of *Pitys antiqua* and *P. primaeva* (Scott, 1923). *Archaeopitys Bastmanii* (Scott and Jeffrey, 1914), from the Waverly Shale of Kentucky, also has medullary bundles in addition to those in a sub-marginal position. In the present instance there is no indication of true medullary bundles, since they are all restricted to marginal and sub-marginal positions, and considerably more of them are marginal than is typical of *Pitys* or *Archaeopitys*.

The primary bundles are much smaller than those described for *Calamopitys* (Scott and Jeffrey, 1914; Thomas, 1935) and *Eristophyton* (Scott, 1902), being

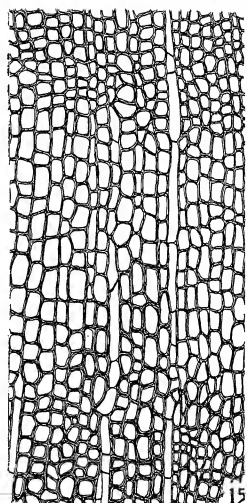
none than that in fig. 8. It is endarch, rounded, somewhat larger, and includes a single parenchyma element at the inner border. $\times 110$.—Fig. 10. A strand dividing tangentially, preliminary to emerging. The left half passed into the secondary wood about 2 mm. above this point, and the right half about 1 cm. above the first. Bundle mesarch. $\times 100$.—Fig. 11. A radial section of secondary wood, showing intercalation of additional elements. $\times 450$.—Fig. 12. Radial sections through three outgoing traces, showing irregularity where leaves were cast off. Note the interruption of secondary wood beneath each trace. CA, callus tissue; P, parenchyma embayment; PRX, primary xylem; ST, short tracheids; ZG, obscure zones of growth.



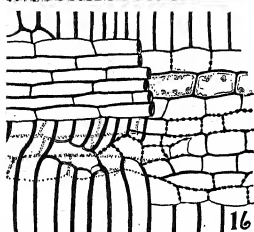
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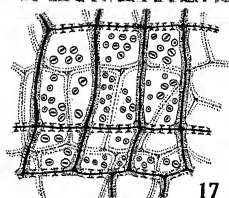
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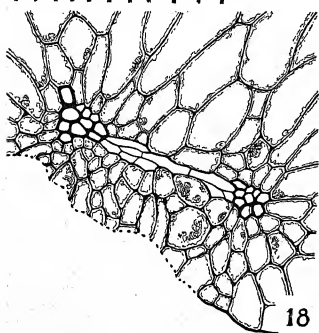
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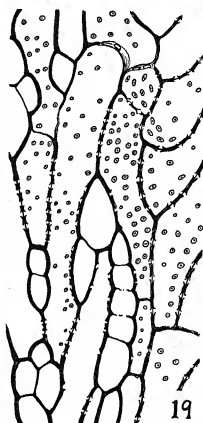
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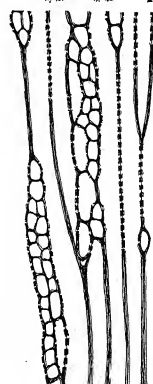
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composed of 6-7 elements where they originate as reparatory strands and having a diameter of about 1 mm. In the sub-marginal position they rarely contain more than 16 elements or exceed 2 mm. in diameter. In this regard they are more reduced than those of *Pitys* and *Callixylon* (Arnold, 1931). Because they are small and the pith large, the strands appear to be widely spaced. There is no tendency for the bundles to become confluent as in *Calamopitys* and *Eristophyton fascicularis* (Scott, 1902).

The small size of primary bundles at the nodes where they turn out as traces and the narrow metaxylem elements are features more typical of Cordaitales than of the Pteridosperms.

Metaxylem of the sub-marginal strands may be transitional from reticulate to pitted, but the multi-seriate pitted condition described for *Eristophyton* (Scott, 1902) appears to be present near the node only and in the outgoing trace, a condition similar to that of *Pitys* (Scott, 1902). The transition from spiral to pitted elements takes place much more quickly than is typical for the Cordaitae, as four or five cells only may intervene in the complete transition. In this respect the condition resembles that found in certain Calamopityeae.

Marginal bundles are mesarch in the nodal region, but at lower levels they lose the centripetal xylem and become endarch like the sub-marginal strands. A similar reduction of centripetal xylem in the lower extension of the primary bundles is characteristic of *Eristophyton* and *Endoxylon* of the Calamopityeae (Scott, 1923, 1924) and is also found in the Cordaitales genera: *Poroxylon*, *Mesoxylon*, and *Parapitys* (Scott, 1923).

A double bundle arrangement where traces enter the secondary wood occurs in *Calamopitys americana* (Scott and Jeffrey, 1914), *Calamopitys foerstei* (Read, 1937), *Dichonia kentuckiensis* (Read, 1936) and *Biligneo solida* (Scott, 1924); but *Eristophyton* and *Endoxylon*, which, of the Calamopityeae, most closely resemble this specimen, have traces which remain single as they pass through the secondary wood. *Pitys* and *Callixylon* are characterized by undivided traces; otherwise the Cordaitales with few exceptions have the double trace. The vertical grouping of paired traces as found in this specimen is unique. Both traces evidently entered the same petiole, but whether or not the superposed arrangement extended through the cortex to the petiole is unknown.

Well out in the wood, a zone of radially arranged secondary elements surround the primary xylem of

each trace, a feature which characterizes *Pitys Dayi* (Scott, 1923), *Archaeopitys* (Scott, 1933), *Callixylon* (Arnold, 1930), and *Calamopitys americana* (Scott and Jeffrey, 1914).

Dense secondary wood with rays mostly uniseriate, characterizes *Calamopitys eupunctata* (Thomas, 1935), *Eristophyton*, and the Cordaitae (Scott, 1923). Tracheids, associated with the rays, have been reported for *Callixylon* (Arnold, 1930) only. The ray tracheids of this stem are different from those of *Callixylon*, in which they occur as regular constituents of the ray, replacing a linear series of parenchyma cells. Here they are found as large, relatively short elements, and apparently occur as transitional cells where new rays were formed by a change in cambial activity or where rays already formed were increased in height or width by involving neighboring cells in the cambium.

The compact wood, arborescent habit, large pith, double traces, obscure growth zones, prevailing endarchy, and reduction of centripetal xylem conform to conditions found in the Cordaitae, but the large number of primary strands and the presence of many sub-marginal bundles are more characteristic of the Pityeae. It can scarcely be assigned to the Pityeae, for as that group is now defined, the leaf trace is single, the bundles are typically mesarch, and the rays are wide.

Two other positions may be considered. One is in the Calamopityeae, as a specialized member of that line which includes *Eristophyton* and *Endoxylon*, where there is a tendency toward a large pith, dense wood, narrow rays, the endarch condition, and arborescent habit. In considering this position, there is disagreement in certain features—i.e., the large number of primary strands, their small size in both marginal and nodal positions, the double leaf trace, and small size of metaxylem elements. Three features which are perhaps least conformable to the calamopityean type are concerned with the primary bundles. In the first instance, they are much smaller than those described for the Calamopityeae. Second, they are composed of metaxylem elements of relatively small caliber, and finally, there is a tendency for the centripetal metaxylem to become radially arranged when in contact with secondary wood. These three features are characteristic of Cordaitales.

The second position, which on the whole seems more tenable, is with the Cordaitales. The two features least conformable to the cordaitae plan are such as would indicate affinity on one hand with the

Plate 3.—Fig. 13-20.—Fig. 13. A tangential section of secondary wood cut about $\frac{1}{4}$ mm. from the pith. Note the relatively wide rays, and the occasional transverse end walls of tracheids. $\times 66$.—Fig. 14. A tangential section cut about 2 cm. from the pith. Note the high central ray and the ray of two large cells to the left of it. At the left center appears a tracheid with transverse wall. The rays are uniseriate or partially biseriate. $\times 66$.—Fig. 15. A transverse section of secondary wood, showing an obscure growth zone of "summer wood" about twelve cells wide, narrow wood rays, and slight radial elongation of wood elements. $\times 66$.—Fig. 16. A radial section, showing a normal ray with adjacent ray of larger cells, some of which have the pitting of tracheids. $\times 50$.—Fig. 17. Short tracheids with transverse end walls on the abaxial side of an outgoing trace. Pits occur on end and radial walls. $\times 250$.—Fig. 18. Anastomosis between two sub-marginal strands. $\times 60$.—Fig. 19. Tangential pitting, as seen on tracheids near an outgoing trace. These pits are smaller and more scattered than elsewhere. $\times 125$.—Fig. 20. A tangential section, showing large tracheid elements associated with normal ray parenchyma. $\times 100$.

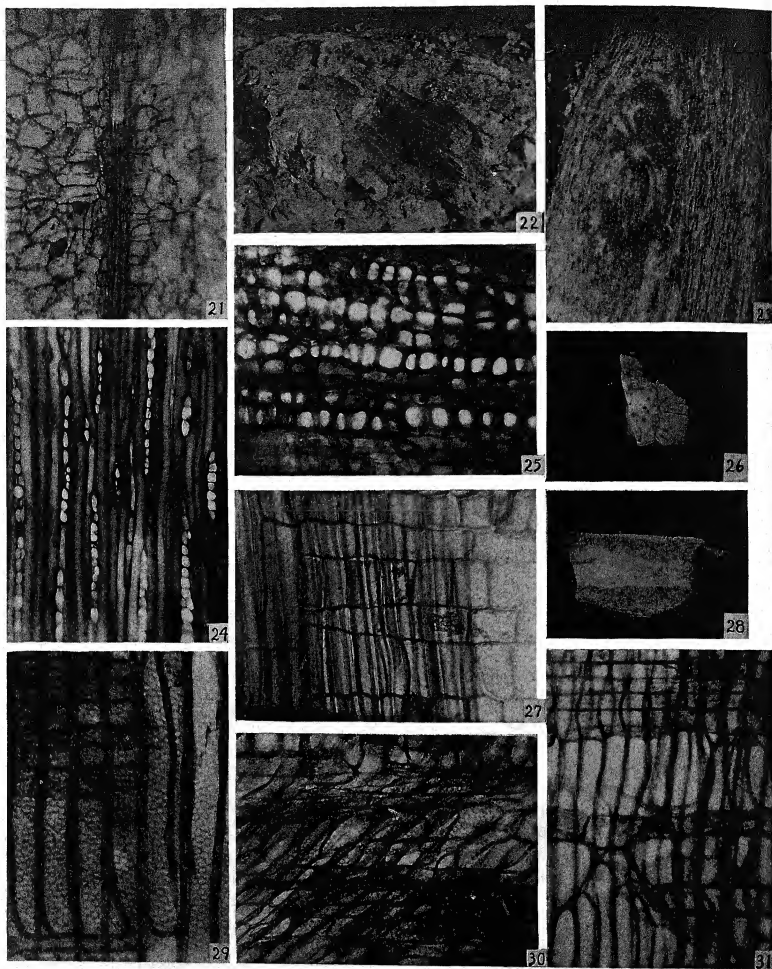


Plate 4.—Fig. 21–31.—Fig. 21. A sub-marginal bundle surrounded by parenchyma cells which resemble a bundle sheath. To the right (near the secondary wood) many pith cells are vertically elongated. $\times 22$.—Fig. 22. The portion of stem found embedded in chert. This part of the stem was badly weathered. Note the limestone core near the center.—Fig. 23. A transverse section of a trace, cut about 2 mm. from the pith. The plane of this surface is vertically oblique to the tangential plane. $\times 17$.—Fig. 24. A tangential section of secondary wood, cut about 3 cm. from the pith. Note the large cells in the ray near the center. $\times 50$.—Fig. 25. A radial section, cut directly opposite the terminus of a trace, showing tracheids that are approximately square. Many of these bear pits on their tangential walls. $\times 130$.—Fig. 26. An end view of the pith with attached secondary wood at the right. The fracture shown here affected most of the boundary between pith and wood. $\times \frac{1}{4}$.—Fig. 27. A radial section, showing the

Pityeae and on the other with the Calamopityeae—namely, the large number of primary strands, many of which are embedded in the pith, and the rapid transition from spiral to pitted elements.

This specimen is interpreted as representing a new genus and species, for which the name *Pycnoxylon leptodesmon* is proposed. It is apparently a primitive member of the cordaitan line that possesses, to an unusual degree, transitional features which indicate affinity with Pteridosperm stock.

DIAGNOSIS.—*Pycnoxylon leptodesmon*, sp. n.—Arborescent; pith large, continuous, uniform, with short and radially elongated cells. Primary strands small, about 45 in number, $\frac{1}{2}$ or more sub-marginal, and tangentially crescentic or elliptical in transv. section; typically endarch, except near and distal to nodes; paired at nodes with vertical grouping; outgoing strands mesarch, and surrounded distally by a radial zone of secondary elements; primary strand of trace may divide in vertical plane. Reparatory strands are apparently single, occasionally anastomose tangentially, and turn out to a marginal position at higher levels. Phyllotaxy undetermined, apparently a complex spiral. Secondary wood dense; obscure zones of growth present; average transverse dimensions of tracheids $42\mu \times 38\mu$. Pits 1–4-seriate, bordered, crowded and hexagonal in larger elements, contiguous and rounded in smaller tracheids or distinctly separated, confined to radial walls except near or in outgoing traces where tangential pitting occurs. Pore, an inclined slit. Rays dilated, with ray cells thick-walled near the pith; radially elongated and muriform distally; 1–3-seriate near the pith, distally, uniseriate or biseriate in part; 1–50 cells high (average 10–15). Occasional tracheids associated with rays;

ray tracheids of larger size than typical ray cells. Ray cell pits smaller than tracheid pits, average about 8 per tracheid on border cells and 6 per tracheid on inner cells.

SUMMARY

This new form is the second stem to be described from the Reed Springs Limestone (Mississippian) of Missouri, a formation which until recently was not known to contain petrified plant remains.

The stem here described represents a small tree with structural characteristics, which, on the whole, are predominantly cordaitan: a large pith, dense wood, paired leaf traces, narrow rays, obscure growth zones, and prevailing endarchy below the node.

There is a resemblance to the Pityeae in the large number of primary strands (about 45, of which one half or more are embedded in the pith), in the character of the primary xylem, and in the radial elongation of pith cells. The manner in which primary strands assume a sub-marginal position, undergo a reduction of centripetal xylem, and become smaller at lower levels is particularly characteristic of *Eristophyton* of the Calamopityeae.

This stem is interpreted as representing a new genus, for which the name *Pycnoxylon leptodesmon* is proposed. It is placed temporarily as a primitive member of the Cordaitae and apparently represents a primitive stock that combines certain characters of the Calamopityeae and Pityeae with features which are prevailing cordaitan.

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origin of a ray composed of cells that are high and short. The large tracheid at the left of the ray has transverse end walls. In rays of this type tracheids sometimes occur. $\times 100$.—Fig. 28. A view of the pith, showing the central mass of chert. $\times 9/10$.—Fig. 29. A radial section, showing crowded pits of the wide tracheids ("spring wood"). $\times 130$.—Fig. 30. A radial section of secondary wood cut near an outgoing trace, showing radially elongated tracheids which are replaced farther out by ray parenchyma. $\times 125$.—Fig. 31. A radial section, showing irregularities in the secondary wood, and pits on the lateral walls of ray cells. $\times 100$.

MICROELEMENTS IN CULTURE-SOLUTION EXPERIMENTS WITH HIGHER PLANTS¹

D. I. Arnon

A PREVIOUS paper (Arnon, 1937) reported that, in a preliminary experiment, molybdenum, chromium, and nickel improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen. This finding suggested that these and other elements, if present in minute quantity, may favorably influence the growth of plants. It was deemed advisable to test this possibility by arranging a number of chemical elements in groups and by observing how the addition of a given group affects the growth of plants in culture solutions. This paper is a report on the observed responses from a group of elements not generally credited with a function in plant nutrition.

EXPERIMENTAL METHODS.—Lettuce and asparagus plants were grown in a basic culture solution of the following composition: $\text{NH}_4\text{H}_2\text{PO}_4$, 0.001 M; KNO_3 , 0.006 M; $\text{Ca}(\text{NO}_3)_2$, 0.004 M; MgSO_4 , 0.002 M.

All the salts except the C.P.-grade ammonium phosphate were purified from heavy metals by a special technique developed in this laboratory—a method to be described by P. R. Stout and the author in a later publication.

In making up culture solutions, distilled water was used throughout. An iron solution containing 0.5 per cent FeSO_4 and 0.4 per cent tartaric acid was added thrice weekly at the rate of approximately 0.6 cc. per liter of culture solution. The number of iron applications was later reduced to one a week. Three supplementary solutions, each containing different elements in minute quantity, were prepared. These solutions were designated as A4, B7, and C13, respectively. In each case, the numeral following the capital letter indicates the number of elements supplied. The A4 solution furnished boron, manganese, zinc, and copper, the four generally accepted as indispensable for plant growth (review by Hoagland, 1937). The basic culture solution supplemented with the A4 solution therefore furnished the plant with a seemingly complete list of essential elements. One cc. of the A4 solution was used for each liter of culture solution (unless otherwise indicated), giving a concentration of 0.5 ppm. B, 0.5 ppm. Mn, 0.05 ppm. Zn, and 0.02 ppm. Cu. The composition of the A4 solution in grams in 1 liter of water was: H_3BO_3 , 2.86; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.81; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.222; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.079.

The supplementary solution designated B7 contained the following seven elements: molybdenum, vanadium, chromium, nickel, cobalt, tungsten, and

titanium—a somewhat arbitrary grouping, based on the consideration that each of these could assume various valence levels and hence, conceivably, might have some part in oxidation-reduction processes within the plant cell. The previously mentioned findings on the role of metals in the nitrogen nutrition of barley (Arnon, 1937) suggested the grouping of these elements.

In making up this solution, care was taken to avoid precipitation. All the constituents were dissolved in N/10 H_2SO_4 . The B7 solution contained the following compounds (milligrams in 10 liters N/10 H_2SO_4): MoO_3 , 85 per cent, 176.4; NH_4VO_3 , 229.6; $\text{Cr}_2\text{K}_2(\text{SO}_4)_4 \cdot 24\text{H}_2\text{O}$, 960.2; $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$, 447.8; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 493.8; $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 179.4, and a titanium solution which was prepared by dissolving 736.6 mg. of TiO : $(\text{COO} \cdot \text{COOK})_2 \cdot 2\text{H}_2\text{O}$ in water, precipitating the titanium with NH_4OH , filtering, dissolving the precipitate in N/10 H_2SO_4 , and combining it with the rest.

One cc. of the B7 solution was used for each liter of culture solution (unless otherwise indicated), giving a concentration of 0.01 ppm. each of Mo, V, Cr, Ni, Co, W, and Ti.

The third supplementary solution, designated as C13, supplied thirteen elements: aluminum, arsenic, cadmium, strontium, mercury, lead, lithium, rubidium, bromine, iodine, fluorine, selenium, and beryllium. One cc. of the C13 solution was used for each liter of culture solution (unless otherwise indicated), giving a concentration of 0.005 ppm. for each of the thirteen elements. Sodium and chlorine, though not singled out, were provided from several sources in these solutions. The sum of A4 + B7 + C13 solutions represents a deliberate addition to the culture medium of 24 elements (or 26 counting Na and Cl), which is similar to the addition of a so-called A-Z solution described by Hoagland and Snyder (1933).

The composition of the C13 solution, in mgm. in 10 liters of water was: $\text{Al}_2(\text{SO}_4)_3$, 317.1; As_2O_3 , 66.1; CdCl_2 , 81.5; SrSO_4 , 104.9; HgCl_2 , 67.7; PbCl_2 , 67.1; LiCl , 305.5; Rb_2SO_4 , 78.1; NaBr , 64.4; KI , 65.4; NaF , 110.5; Na_2SeO_4 , 119.4; $\text{Be}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, 1,037.0.

The plants were germinated in white sand and, upon attaining a size convenient for transplanting, were placed in tanks with the culture solution. The type of tanks, the method of supporting the plants, and the method of aerating the culture solution have already been described (Hoagland and Snyder, 1933; Arnon, 1937). Forced aeration by means of all-glass Pyrex aerators was used throughout.

The asparagus plants were grown in the greenhouse from May 18 to September 7. Twelve plants, representing each treatment, were placed in tanks of 40

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The writer is indebted to Prof. D. R. Hoagland for reading the manuscript and for making the facilities of the Division of Plant Nutrition available for this investigation, to Mr. P. R. Stout for furnishing the purified salts and reading the manuscript, and to Mr. S. B. Johnson for valuable technical assistance.

liters' capacity. The culture solution was changed twice during the experiment. On June 5 the A4 solution was added to the respective tanks, and on June 25 the B7 and C13 treatments were begun. The harvested plants were separated into shoots and roots. The shoots were weighed directly, whereas the roots were washed in distilled water and centrifuged as previously described (Arnon, 1937) before weighing.

Lettuce plants of the Imperial D variety were placed in tanks with culture solution on June 1 and grown outdoors till August 3. This period was free from rain except for a shower in the first week, after which the culture solution was changed. The second change of the culture solution occurred five weeks later. Sixteen plants were placed in tanks of 115 liters' capacity. A number were removed on July 9 to avoid crowding, but at least nine were left for each treatment. Forced aeration was begun on June 18. The quota of microelements was added in three applications. On June 25, $\frac{1}{4}$ cc. of the A4 and $\frac{1}{2}$ cc. of the respective B7 and C13 supplementary solutions were added for each liter of culture solution. This application was repeated on June 25; on July 13, $\frac{1}{2}$ cc. of the A4 and $\frac{3}{4}$ cc. of the B7 and C13 solutions were added, completing the addition of 1 cc. of each of the three supplementary solutions to a liter of culture solution in the respective treatments. When the solution was subsequently changed, the full quota of microelements was added in one application. Fresh weights of shoots and roots were determined in the same manner as for the asparagus plants.

RESULTS AND DISCUSSION.—The fresh weights of the asparagus plants grown with the same basic culture solution to which different groups of microelements were added are given in tables 1 and 2.

Both the asparagus and lettuce plants grown in culture solution to which no microelements were added made very poor growth and showed symptoms of severe deficiency. The lettuce leaves were stunted and pale, and the roots were short and distinctly limited in growth. A similar general effect was noted in the "no microelements" treatment with the asparagus plants.

The poor growth of plants in this treatment confirmed the impression, based on other trials, that asparagus and lettuce plants could not develop normally unless at least boron and manganese were added to the culture solution. Results from unpublished experiments indicated that so far as zinc and copper were concerned, the impurities derived from the ordinary distilled water and metal culture tanks used went far towards supplying the needs of the plant, whereas well defined deficiency symptoms were produced by omitting either manganese or boron from the culture solution. When no microelements were added to the culture solution, the deficiency symptoms of both the asparagus and lettuce plants resembled those of the manganese more than of the minus boron plants.

Judging from tables 1 and 2, the growth of the plants was markedly assisted by the supplementary

A4 solution which supplied boron, copper, manganese, and zinc. Obviously, the symptoms of malnutrition observed when no microelements were added to the culture solution were traceable to the deficiency of some or all of the four elements supplied by the A4.

TABLE 1. *Effect of adding different groups of microelements on the growth of asparagus plants in culture solution. Average fresh weight of plants in grams.*

Microelements added	Shoots	Roots
None	16.2	12.8
A4 (B, Zn, Mn, Cu)	88.2	38.2
A4 + B7 (Mo, Ti, V, Cr, W, Co, Ni)	118.1	81.3
A4 + B7 + C13 (Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be) ...	121.7	74.5

TABLE 2. *Effect of adding different groups of microelements on the growth of lettuce plants in culture solution. Average fresh weight of plants in grams.*

Microelements added	Shoots	Roots
None	71.4	14.5
A4 (B, Zn, Mn, Cu)	105.7	22.0
A4 + B7 (Mo, Ti, V, Cr, W, Co, Ni)	1,068.3	188.6
A4 + B7 + C13 (Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be) ..	984.4	196.2

The beneficial response obtained by adding the A4 solution confirmed the already established importance of boron, manganese, copper, and zinc in the nutrition of higher plants. The growth of plants was markedly assisted, however, by the further addition of the supplementary B7 solution, comprising seven elements not generally identified with a function in the nutrition of higher plants. This effect was particularly striking in lettuce plants, giving a tenfold increase in the fresh weight of the harvested plants (table 2 and fig. 1). The plants treated with the B7 solution were dark green and showed vigorous growth of root or shoot. When the effect of the B7 on lettuce plants was again tested in the fall season, a distinct improvement over the growth of plants supplied only with the A4 supplementary solution was again noted. This beneficial effect of the seven elements in the B7 was relatively less pronounced, however, in the fall than in the summer. The increase in the growth of asparagus plants resulting from the addition of the B7 solutions is indicated in table 1. A similarly beneficial effect from adding the B7 is noted in another asparagus experiment now in progress.

The further addition of thirteen more elements supplied by the C13 solution produced no measurable effect on either the lettuce or the asparagus plants. The plants in this treatment did not differ in growth habits from those supplied with only the A4 plus the B7 supplementary solutions. This finding is interpreted as not disposing of the question as to whether

one or more of the elements contained in the C13 group play some part in the nutrition of the plants investigated. The conclusion is drawn that with the experimental technique employed in this investigation no measurable effect was produced by the thirteen elements present in the C13 solution, but the possibility of obtaining different results by means of a more refined technique is not excluded. It is further concluded, from the beneficial effect of the B7 treatment, that the seven elements in this group have supplemented qualitatively or quantitatively the impurities of microelements already present in the culture medium, other than boron, manganese, zinc, and copper. The history of the discoveries of the essential nature of these four elements illustrates the importance, in all investigations on microelements, of care in experimental procedure—rigid purification of chemicals and water and selection of suitable culture vessels.

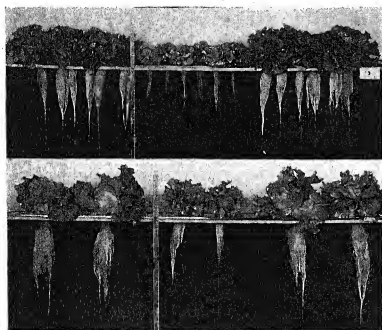


Fig. 1. Effect of microelements on lettuce plants grown in culture solution: Group in the center has received B, Mn, Zn, and Cu. Group on the left has received these four plus Mo, V, W, Ti, Cr, Ni, and Co. Group on the right has received all of these plus Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, and Be.—A (above), plants 40 days in culture solution. B (below), plants 64 days in culture solution.

In this investigation, in order to simplify the complex experimental task of studying the effect of a large number of elements in minute quantity on plant growth, the elements studied were divided into more or less arbitrary groups. With this approach, the growth of lettuce and asparagus plants was significantly improved by the addition of one group of

seven, but not by the further addition of another group containing thirteen elements. The experimental finding that the joint addition of molybdenum, chromium, titanium, tungsten, cobalt, nickel, and vanadium has produced large and favorable effects on the growth of lettuce and asparagus plants, is considered highly significant in itself, quite apart from the question of what elements in this group are effective in plant nutrition. The observed influence of the supplementary B7 solution on plant growth is offered in support of the suggestion that the list of essential elements, as it stands today, is incomplete.

Of the seven elements contained in the B7 solution, several have been associated in recent investigations with biological phenomena. Burk and Horner (1935) and Bortels (1937a) found molybdenum and vanadium effective in promoting nitrogen fixation and growth of *Azotobacter*. Bortels (1937b) reported that molybdenum and vanadium applications stimulated the growth of legumes in sand cultures.

In investigating the nutrition of the strawberry plant, Hoagland and Snyder (1933) found that plants receiving a supplementary solution consisting of 26 microelements (solution B) surpassed those receiving a supplementary solution of 12 (solution A), the latter containing among others boron, manganese, zinc, and copper. Molybdenum, vanadium, tungsten, and chromium, which are common to Hoagland and Snyder's solution B and to the supplementary B7 solution used in this investigation, were not included in solution A. Robbins and others (1936) have recently reported marked beneficial effects on the growth of excised root tips as a result of adding agar ash or filter-paper ash to the culture solution. In the opinion of the investigators these beneficial effects are attributable to one or more elements other than boron, manganese, zinc, and copper.

SUMMARY

Lettuce and asparagus plants were grown in water-culture solutions supplemented with different groups of elements in minute quantity.

A marked beneficial response was obtained from the joint addition of boron, manganese, zinc, and copper, a result confirming previous findings.

A further significant increase in the growth of lettuce and asparagus plants was obtained by adding a group of seven elements (molybdenum, vanadium, titanium, tungsten, chromium, nickel, and cobalt)—a fact suggesting that one or more of these elements plays a part in the nutrition of higher plants.

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EXTENSIBILITY OF CELL WALL MATERIAL IN INDOLE-3-ACETIC ACID

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SINCE HEYN (1931) pointed out that auxin increased the extensibility of the cell wall (including both plastic and elastic extensibility), the question of the mechanism of its action has been the center of much discussion. Two viewpoints on this subject are possible: either that the auxin acts directly on the cell wall or that it acts indirectly through the cytoplasm. The evidence for the latter viewpoint is well summarized by Went and Thimann (1937). On the other hand, Robbins and Jackson (1937) performed experiments which suggest that the action of growth hormone is on the cell wall directly. In these experiments it was found that when 2 per cent indole acetic acid in lanolin was applied to living or dead

were suspended in a dark room with a 120-gram weight attached to each. Three pieces were untreated, three were rubbed with lanolin only, three with 2 per cent indole-3-acetic acid in lanolin, and three with 2 per cent acetic acid in lanolin.

Four pieces of hemp, approximately 1000 mm. long and each consisting of six single fibrils, were subjected to the same treatment. To eliminate the possibility of differences in water content, lanolin from the same container was used to make the mixtures mentioned above. These mixtures were then used in all the subsequent experiments.

The increase in length of the material after three weeks is shown in table 1.

TABLE 1. *Lanolin treatments on cotton and hemp.*

Treatment	Cotton				Hemp	
	% total length increase in 21 days				% total length increase in 23 days	
No treatment	41,	38,	14	31	0.00	
Lanolin only	54,	46,	51	50	0.00	
Lanolin—2% indole acetic acid	60,	46,	41	49	0.19	
Lanolin—2% acetic acid ..	66,	60,	55	60	0.18	

cell wall materials it caused an increase in their extensibility. It was also found that the same treatment decreased the extensibility of both fresh and dried root cell wall materials. However, they point out that the effects obtained are not necessarily specific for the growth hormone used (indole-3-acetic acid) as no controls of pH were used; and furthermore, since the water content was not known to be the same in the lanolins used, the results attributed to growth hormone may have been partly due to such differences. In view of these circumstances and because of the importance of theoretical conclusions regarding growth mechanisms which might be based on such data, the experiments were repeated with as nearly identical material as possible. Several new experiments were also devised.

EXPERIMENTS AND RESULTS.—I. Twelve pieces of No. 8 cotton thread approximately 2100 mm. long

The indications are that in various mixtures applied to cotton thread, lanolin alone is the cause of the increased extensibility, whereas in hemp the increased extensibility is due to the organic acid in the lanolin.

II. Strips of different materials were rubbed with lanolin, lanolin-2 per cent (by weight) indole acetic acid, lanolin-2 per cent acetic acid, and lanolin-2 per cent water. They were 7.5-15 cm. long, depending on the material. Within any one group they were the same length. Each strip in turn was fastened in a horizontal position on a wooden block with 2.5 cm. of its base abutting on a piece of wood. The remainder of the strip extended horizontally and free in the air. A small lead rider was attached to the end of each piece, and the angle through which each strip bent from the plane of the wooden block was measured by a protractor. The pieces were then laid on a table, in diffuse light, at a temperature of 23°C. After a period of time the angle of bending of each

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TABLE 2. *Lanolin treatments on stem and root materials.*

			Average difference in degrees between original & final curvature (& stand. error) in:			
Material	Number of strips	Hours of treatment	Lanolin	Lan-indole acetic	Lan-acetic acid	Lan-water
STEM:						
<i>Ambrosia</i>	5	12	2 ± 1.2	—2 ± .7	—4 ± .9	.2 ± .4
Potato	4	30	0 ± .6	0 ± .4	0 ± 0	.25 ± 1.3
Cardboard	5	14	.6 ± .12	.8 ± .09	.2 ± .05	1.0 ± .5
ROOT:						
Carrot	5	34	7.6 ± 3.3	3.6 ± 3.0	3.4 ± 2.0	2.2 ± 1.0
Willow	4	30	1.7 ± 1.1	.25 ± .1	.25 ± .1	—1.0 ± .7
<i>Chenopodium</i>	1	17	3.0	1.0	4.0	0
Corn Branch	10	20	—6 ± .8	—1 ± .1	—4 ± .6	.3 ± .5
Corn Brace	4	18	2.0 ± 3.3	2.8 ± .8	2.8 ± .9	2.5 ± .7
<i>Ambrosia</i>	4	13	4.5 ± 3.5	2.0 ± 3.4	4.5 ± 4.2	.3 ± 1.2
Onion	8	29	5.5 ± 3.0	5.1 ± 2.2	6.5 ± 2.2	6.6 ± 3.2
<i>Pistia stratiotes</i>	4	32	24.0 ± 7.7	24.0 ± 10.5	17.0 ± 9.0	27.0 ± 1.1
<i>Ludwigia</i> sp.	5	32	2.0 ± .7	3.2 ± 1.4	3.8 ± 1.6	7.0 ± 2.8
Pea	2	20	7.5	13.0	12.5	14.0

strip was again measured. Except in the cases of the cardboard strips, the fresh *Chenopodium album* roots, and the *Ambrosia psilostachya* stems and roots, all the material had been previously dried in an herbarium press. The results are presented in table 2.

An inspection of these data shows that there are no constant, specific effects of any of the treatments for the material used.

III. In view of the great variability found in the experiments carried out according to the methods of Robbins and Jackson (1937), it was decided to adopt other methods by which more consistent results might be obtained. It was desirable to have (1) a means of controlling the water content of the material used and (2) a more accurate method of measuring the extensibility. Accordingly the material was subjected to experimental conditions in water solutions, thus eliminating any differences in water content of the original material. The extensibility was measured accurately to .1 mm. by means of a horizontal microscope.

The apparatus consisted of a vertically mounted glass tube, 60 cm. long and 1.6 cm. in diameter. It was possible to secure one end of the material under investigation at the bottom by means of a wire hook. The other end was fastened to a fine copper wire which ran up and over a pulley (having but little friction) and down to a pan for weights. Supporting the weight pan was a vertically movable stage mounted on a screw in order to lower the weight pan gradually and thus avoid a sudden application of the stretching force which otherwise might break the material. At the top of the glass tube a millimeter scale was mounted parallel to the copper wire. Any change in length of the material was read with the microscope by observing movement along the scale of an indicator which was perpendicularly attached to the wire.

The bottom of the tube was equipped with a drain to facilitate changing solutions. The ramie, cotton, and artificial silk threads were secured with small loops to the wire hooks at the top and bottom. The root material was cemented to wire loops by means of hard De Khotinsky cement. These loops were then easily placed on the wire hooks in the tube.

Two-thread strands of ramie, 35 cm. long, and single strands of No. 8 cotton thread the same length were stretched by a 100-gram weight. The solutions used were water 0.001 N HCl and .2 per cent indole acetic acid (pH 3.3). For ramie the results expressed as percentage total length increase after 1,000 minutes were: water, .75; .001 N HCl, .62; .2 per cent indole acetic acid, .50. The results for cotton are given for the first 10-minute period since only .5 per cent of the percentage total increase in length occurred during the next 99 hours. They were: water, 2.78 per cent; 0.001 N HCl, 2.06 per cent; .2 per cent indole acetic acid, 1.98 per cent; .2 per cent acetic acid, 2.08 per cent. Because of the slight extensibility of these materials, more statistically significant data were not sought. From these values there is no indication of any specific increase of the extensibility due to pH or the presence of indole acetic acid.

IV. Since ramie in particular is known to have nearly perfect orientation of the cellulose micelles parallel to the longitudinal direction of the fiber axis, it was thought desirable to investigate some material with less complete micellar orientation. For this purpose artificial silk (regenerated cellulose) was used. The strands consisted of two threads, each of which was composed of fifty fibrils. They were stretched according to the manner described above. The results are presented in table 3.

The figures show that indole acetic acid does increase the extensibility of artificial silk, but the effect

is not specific, as shown by the fact that other organic acids act similarly.

This phenomenon can not be explained on the basis that the organic acids esterify the free hydroxyl groups on the 2,3,6 carbon atoms of the glucose rings of the cellulose of artificial silk. This is shown by the following experiment: Strands of artificial silk

TABLE 3. *Extensibility of artificial silk in various solutions.*

Solution	Average percentage total length increase in 10 minutes with stand. error
Water	13.5 \pm 2
0.001 N HCl, pH 3.0	13.7 \pm 2
2% Acetic acid, pH 3.3	16.1 \pm 5
2% Indole acetic acid, pH 3.3 ..	16.2 \pm 4
1% Trans Cinnamic acid	15.4 \pm 1
2% Oxalic acid	15.6 \pm 4
2% Propionic acid	14.5 \pm 3

were weakly acetylated by treating with acetic anhydride. Their extensibility was determined by stretching them with a 100-gram weight for 10 minutes. It was found to be 15.6 per cent, or within the same range as that for 2 per cent acetic acid. Furthermore, allowing the artificial silk to soak in water, 0.001 N HCl, 2 per cent acetic acid, or 2 per cent indole acetic acid for 21 hours and then stretching for 10 minutes gave values similar to those obtained above. They were as follows (data as percentage total length increase): water, 13.4; 0.001 N HCl, 13.9; 2 per cent indole acetic acid, 16.2; 2 per cent acetic acid, 16.2.

It may be concluded that certain non-growth-promoting organic acids increase the extensibility of artificial silk over that in water or in .001 N HCl. The solutions are effective only during the time the stretching force is acting. This is shown by the fact that pretreatment with the solutions in question is without effect.

V. Roots, 30 cm. long, from an onion bulb growing in water and from the water plants *Pistia stratiotes* and *Ludwigia* sp. were thoroughly dried in an herbarium press. The roots were less than 2 mm. in diameter. They were stretched (with equal forces) in water, in 2 per cent indole acetic acid, and in 2 per cent acetic acid. The results, in the order noted for the solutions, were (as percentage total length increase in 10 minutes): onion, 2.52, 3.10, 3.93; *Pistia stratiotes*, 4.20, 4.68, 4.64; *Ludwigia* sp., 8.70, 9.80, 12.20. It may be seen that there is no indication that indole acetic acid has caused a decreased extensibility of root walls.

Fresh water cultured onion roots were stretched in the manner described in section III. A weight of 20.8 grams was used. The roots were selected for uniformity of diameter and were about 13 cm. long. Since it was found that the slope of the extensibility-

time curve was constant after the first 77 minutes, the readings presented were taken at that time. The results (expressed as percentage total length increase with standard error) were: water, $3.97 \pm .09$; 2 per cent acetic acid, $6.60 \pm .68$; 2 per cent indole acetic acid, 8.50 ± 1.50 . From these data it is clear that the root material in 2 per cent indole acetic acid and in the 2 per cent acetic acid had an increased extensibility over that in water. As the values obtained for the acetic acid and the indole acetic acid do not show a significant statistical difference, the effect observed is probably a general one for organic acids at pH 3.3.

Discussion.—It was found difficult to draw definite conclusions regarding cell-wall extensibility based on experiments involving dried stem and root materials with lanolin applied to them. The results of such procedures showed variability and inconsistency. This was probably due to individual differences in the materials. With an improved technique, in one case (that of artificial silk) statistically significant differences were obtained. If this be accepted as an example of stem cell wall material, then 2 per cent indole acetic acid does cause a small increase in the extensibility, but likewise so do other organic acids known not to be growth-promoting substances in plants.

No indication of a decreased extensibility of root wall material as suggested by Robbins and Jackson (1937) was found. On the contrary, it was observed that root material had an increased extensibility in 2 per cent indole acetic acid as well as in 2 per cent acetic acid. An "acid effect" has been noted by Bonner (1934) who found that *Avena* coleoptiles at a pH of 4.1 have a much greater plasticity than those at a pH of 7.1.

While the results given in this paper are concerned with concentrations (.2 per cent or $1.14 \times 10^{-2}M$.) of hetero-auxin much higher than exist physiologically, it is interesting, nevertheless, to compare them with the effects of the lower ones. Auxin at a physiological concentration has been shown by Heyn (1931, 1934) to increase the plastic extensibility of *Avena* coleoptiles and *Lupinus* hypocotyls. Söding (1933) has found a similar action of physiological concentrations of auxin on flower stalks. Amstrong (1937) has observed that a concentration of hetero-auxin between 10^{-6} to $10^{-8}M$ increased the extensibility of roots of *Vicia Faba*. Concentrations of 10^{-8} to $10^{-3}M$ caused a decrease in their extensibility, while a concentration of $10^{-2}M$ was found, in agreement with the work presented here, to result in an increased extensibility of non-living roots.

SUMMARY

The experiments of Robbins and Jackson (1937) concerning the extensibility of stem and root walls were repeated in as nearly an identical manner as possible and found to give no conclusive evidence as to the effect of growth hormone upon them.

Artificial silk was found to have an increased extensibility in certain organic acids known not to be

growth substances, as well as in .2 per cent indole acetic acid.

Onion roots were found to have an increased extensibility in .2 per cent indole acetic acid and in .2 per cent acetic acid.

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A FURTHER STUDY OF CATENARIA¹

J. S. Karling

SINCE THE time *Catenaria* was first described and figured by Villot (1874) and Sorokin (1876) and subsequently rediscovered by Dangeard (1885), investigators of this genus have reported a marked difference in size, shape, structure, and behavior of the zoospores of what is now known as *C. anguillulae*. These differences are so pronounced that Fischer (1892), Constantineanu (1901), and E. J. Butler (1928) have raised the question as to whether or not more than one species is now included under this binomial. In the course of my cytological study of *C. anguillulae* bearing directly on this problem, another distinctly different species was discovered in dead algal cells. This species, for which I am proposing the name *C. sphaerocarpa*, is distinguished primarily by large spherical zoospores with a single, conspicuous refractive globule and a characteristic type of swimming, brown resting spores, and predominantly spherical zoosporangia. *Catenaria anguillulae*, on the other hand, has oval swarmspores with several small refractive granules, hyaline resting spores, and usually uiform zoosporangia.

CATENARIA sphaerocarpa nov. sp.—Thallo praecipue polycentrico, aliquando monocentrico. Zoosporangii hyalinis, levibus, semper fere globosis, 8–50 μ , ovatis, 8 \times 10 μ – 30 \times 33 μ , fusiformibus aliquando, 7 \times 14 μ – 15 \times 25 μ , uno usque ad tribus tubulis dimissionis, directis, flexis vel irregularibus, 5 – 187 μ long. \times 2.5 – 4 μ dia. praeditis, tubulis superficie cellulae hospitis terminatis vel 3–125 μ ultra extensis. Zoosporis globosis, 4–4.8 μ , magno hyalino refracto globulo praeditis; cilio c. 25 μ long.; ex ordine singillatim emergentibus atque post moram brevissimam natantibus. Isthmis inter sporangia longitudine diversis, raro inflatis, fusiformibus; rhizoideis numerosis, late patentibus, ramosis, isthmis atque lateribus atque sporangiorum finibus locatis. Spor. perdurantibus fere globosis et ovatis, 10–25 μ dia., aliquando fusiformibus et elongatis, fusco pariete

1.5–2.5 μ crassitudine, atque aequo protoplasmate granuloso completis; similibus zoosporangii manifeste evolutis; germinatione incompta.

CATENARIA sphaerocarpa nov. sp.—Thallus predominantly polycentric, occasionally monocentric. Zoosporangia hyaline, smooth, usually spherical, 8–50 μ oval, 8 \times 10 μ – 30 \times 33 μ , and sometimes spindle-shaped, 7 \times 14 μ – 15 \times 25 μ , with 1–3 straight, curved, or irregular exit tubes, 5–600 μ long and 2.5–4 μ in diameter, which may end flush with the surface of the host cell or extend 3–200 μ beyond it. Zoospores spherical, 4–4.8 μ with a single, large hyaline refractive globule; cilium approximately 25 μ long; emerging singly in succession and after a momentary pause swimming away. Isthmuses between sporangia of variable length, rarely inflated and spindle-shaped. Rhizoids numerous, well developed and branched, arising from the isthmuses as well as from the sides and ends of the sporangia. Resting spores usually spherical and oval, 10–25 μ , occasionally spindle-shaped and elongated with a heavy brown wall 1.5–2.5 μ thick, and an evenly granular content; apparently developed in the same manner as the zoosporangia; germination unknown.

Saprophytic in dead and cooked cells of *Hydrodictyon reticulatum*, *Chara coronata*, *Nitella flexilis*, *Cladophora glomerata*, *Pithophora* sp., *Spirogyra crassa*, *Elodea canadense*, and root tips of *Zea mays* and *Allium cepa* in New York City.

DEVELOPMENT AND STRUCTURE OF *C. SPHAEROCARPA*.—The zoospores of *C. sphaerocarpa* are spherical and hyaline and vary from 4–4.8 μ in diameter. As is shown in figures 2 and 13, they contain a single clear, highly refractive globule, 0.8–1.2 μ , in the posterior part of the body and a large opaque and somewhat crescentic structure or region which bears close resemblance to the extranuclear cap of *Cladochytrium replicatum*. In living material the nucleus is not readily visible, so that it is difficult to determine its exact relation to this more opaque body. The cilium

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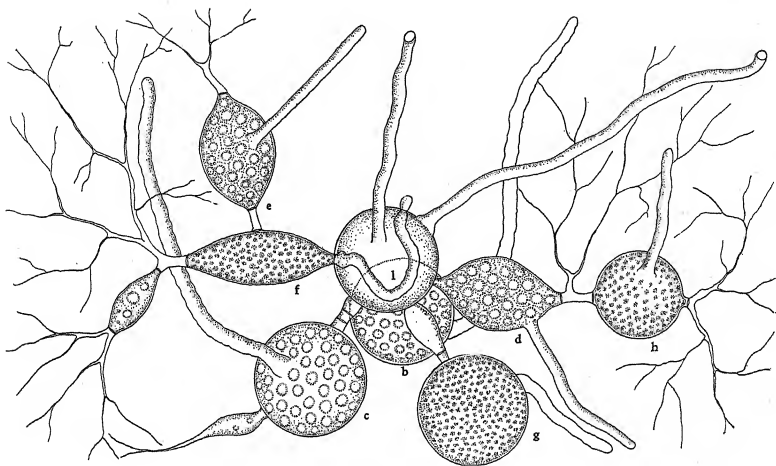


Fig. 1. An extensive thallus of *C. spheroecarpum*.

is approximately 25μ long and often extends straight backwards when the spores are at rest.

Their method of swimming is quite unlike that of the zoospores of *C. anguillulae* and more characteristic of the monocentric rhizidiaceous chytrids. They jerk and dart about a great deal in swimming, come to rest momentarily, and then dash off again in another direction. As far as my observations go at the present time, however, the zoospores do not attain the degree of activity of the spores of *Diplophlyctis* for instance, nor do they travel great distances. They usually come to rest in about 30 to 45 minutes in the vicinity of the infected host cell. Shortly after coming to rest (fig. 14), the internal structure of the spores becomes more homogeneous optically, and the conspicuous refractive globule frequently appears to lie in a clear space. This appearance, however, is doubtless an optical illusion due to the diffraction or bending of the light waves around the globule. After a few hours one to several small granules usually appear in the homogeneous cytoplasm, and the zoospores which do not degenerate or germinate may remain in this condition for as many as 8 hours without undergoing any marked change except for an increase of $1-3\mu$ in diameter. Occasionally swarm-spores may be found in pairs, as is shown in figure 15, but although such spores have been kept under observation for more than 10 hours, no fusion has yet been seen.

The zoospores may germinate in water (fig. 3, 16) and form a comparatively thick germ tube, but so far none has been found to develop beyond this stage. Germination occurs fairly abundantly on various host

cells, and in the process the germ tube pushes through the wall into the lumen on the cell. I have been unable so far, however, to determine with certainty whether the primary sporangium begins as an enlargement of the germ tube after it has branched, as in *Diplophlyctis*, *Endochytrium*, etc., or develops otherwise, but I am of the opinion that its rudiment is formed very shortly after the tube enters the host cell. Figure 4 shows a young thallus in which the incipient primary sporangium is subtended by a short stalk with two main branches of a rhizoid-like absorbing system. The cytoplasm of the young sporangium is usually very vacuolate at this stage and contains one to several refractive globules of varying sizes. Very often a large central vacuole occupies the greater portion of the sporangium. Young thalli in this stage of development are strikingly similar to those of *Endochytrium operculatum* except for certain optical properties of the cytoplasm and may be readily mistaken for this fungus. The rudiments of the absorbing system, secondary sporangia, and the remainder of the rhizomycelium are not always oriented on the base of the primary sporangium at this stage but may arise from the sides as well (fig. 1, 8).

The same thallus five hours later is shown in figure 5. The primary sporangium has increased considerably in diameter, and the two main axes of the absorbing system have enlarged into elongated spindle-shaped structures, which may now be recognized as the rudiments of secondary sporangia. Figure 6 shows the same thallus 13 hours later. The primary sporangium has attained mature proportions and formed a comparatively short exit tube. What was

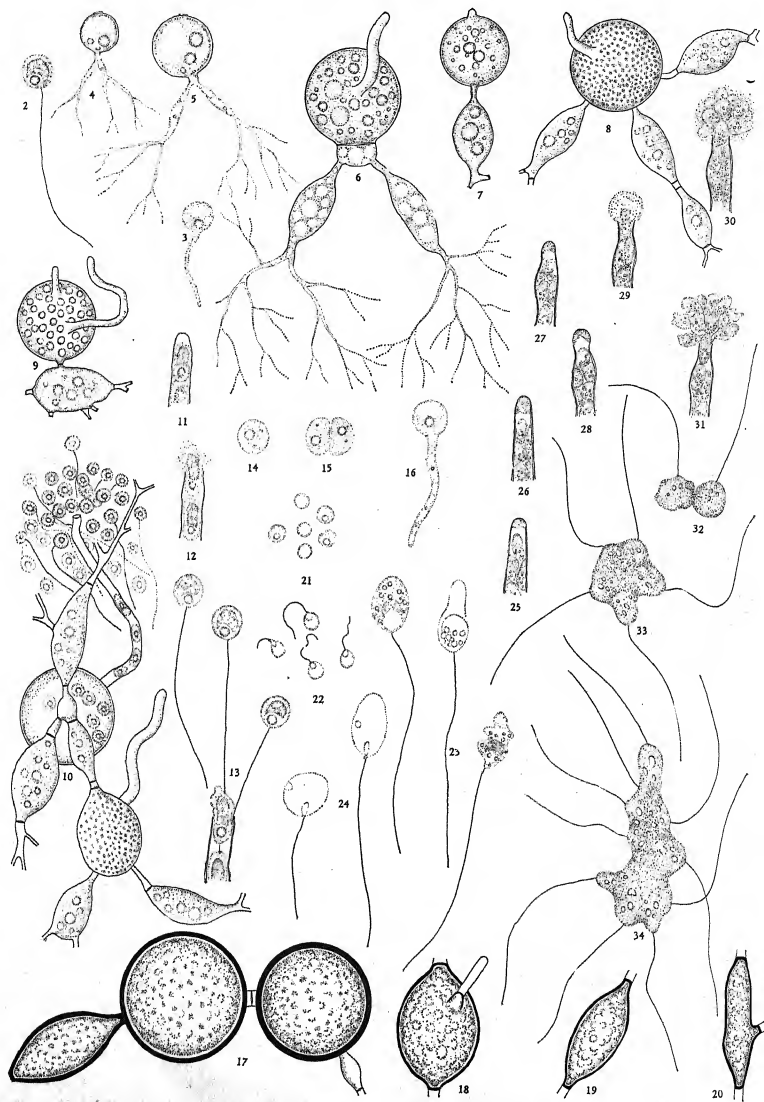


Fig. 2-34. *Calenaria sphaerocarpum* and *C. anguillulae*.

formerly the short stalk on which the absorbing system was oriented in figures 4 and 5 has now become greatly enlarged, vesicular, and apophysis-like, and is delimited from the primary sporangium by a cross wall. Such cells or enlargements may be lacking entirely, in which cases the primary and secondary sporangia are separated by narrow isthmuses (fig. 8) as in *C. anguillulae*. The two secondary sporangia shown in figure 6 have become broadly spindle-shaped and highly vacuolate and are still continuous with the rhizoids and the apophysis-like enlargement at the base of the primary sporangium. The cytoplasm of this sporangium is dense and filled with numerous large suspended and refractive globules. At this and even earlier stages these globules may sometimes attain a diameter of 6.6μ .

The development of the thallus shown in figure 6 was followed through to maturity. The primary sporangium emitted its zoospores after 26 hours and partially collapsed, while the two secondary sporangia became delimited from the remainder of the thallus by cross walls and reached almost the same spherical dimensions as those of the primary one. Only three sporangia were thus formed in this thallus, so that the rhizomycelium was accordingly rather limited in extent. Occasional monocentric thalli have been found, as in *C. anguillulae*, and in several cases only two sporangia were developed, as is shown in figure 7 and 9. In large host cells such as the internodes of *Nitella* the thallus or rhizomycelium is usually more extensive. Figure 1 shows a thallus from this host which developed 8 sporangia. The sequence of their development and maturity as evidenced by the time of zoospore emission is indicated in alphabetical order. The degree of maturity of the secondary, tertiary, quaternary, etc., sporangia at any given time is usually in proportion to their distance from the primary sporangium and the sequence in which they were formed. Exceptions, however, may occasionally occur. In figure 1 the secondary sporangium *f*, for instance, emitted its zoospores later than *e*, although its rudiment was laid down earlier. It is significant to note in this connection that the exit tube of this sporangium passes into and then out of the empty primary sporangium.

Figure 1 illustrates in addition the space relationship of the primary, secondary, tertiary, etc., sporangia. They are usually but not always separated at maturity by hyaline, isthmuses of varying length and diameter. Occasionally these may be somewhat inflated and have the appearance of the unicellular spindle organs of *C. replicatum*. As to shape, the mature sporangia are predominantly spherical, although slightly oval, ellipsoidal, and broadly spindle-shaped ones may also occur. Sometimes sporangia may develop between the layers of the thick softened walls of *Cladophora*, and in such instances they are usually somewhat irregular in shape. As a rule the sporangia are not at all uteriform as in *C. anguillulae*, and when the two species occur side by side in the same host, they may be readily distinguished

by this difference in shape. As to size, the spherical sporangia are from 8 to 50μ in diameter, while the oval and spindle-shaped ones vary from $8 \times 10\mu$ – $30 \times 33\mu$ and $7 \times 14\mu$ – $15 \times 25\mu$. The exit tubes vary from 1–3 in number, 5–600 μ in length, and 2.5–4 μ in diameter. Their diameter is usually fairly uniform throughout, and they rarely taper from base to apex as in *C. anguillulae*. They may be straight, curved, or irregular and are usually undulating in contour. They may end flush with the surface of the host cell or extend from 3–200 μ beyond it. The rhizoids are usually well developed, as is shown in figure 1, branch rather freely, and may often extend for a distance of more than 75 μ . They may occur on the isthmuses as well as on the sides and ends of the zoosporangia.

In the maturation of the sporangium from the stage shown in figures 6 and 7, the larger refractive globules apparently break up gradually into smaller and smaller units and become highly dispersed, so that the protoplasm eventually has the appearance shown in figure 8. Concomitant with this increase in degree of dispersion, the protoplasm becomes less refractive and appears finely greyish, granular, and non-vacuolate. This condition and appearance may persist for several hours, but as time goes on, minute refractive bodies begin to appear again. This doubtless comes about by coalescence in the manner which I (1938) have recently described in *N. appendiculatum* and *E. digitatum*. Coalescence continues further until a more or less definite number of large spherical highly refractive globules are formed, as is shown in figure 9. At what stage in maturation cleavage occurs is difficult to determine in living material, but it none the less takes place in such a manner that one refractive globule is included in each zoospore initial.

The dehiscence of a large number of sporangia has been carefully observed at different time intervals to determine the method of zoospore emission with the view of comparing it with the process in *C. anguillulae*. In a few cases, as is illustrated in figure 10, the swarmspores emerged one by one and came to rest in a loose and scattered mass at the mouth of the exit tubes. Such masses were kept under observations for a long time to determine the subsequent behavior of the zoospores. The latter never became very actively motile, but merely jerked and spun around in the mass and eventually degenerated. Such observations have led me to the conclusion that this is not the normal type of behavior. In the majority of sporangia so far studied, the tip of the exit tube (fig. 11) gradually deliquesced, and at the moment of rupture a small round mass of slightly viscid sporangial fluid escaped. This mass usually becomes hydrated very quickly, and its outline disappears after a few seconds. Unless one observes the exact moment of rupture, it may be overlooked completely. The initial spore in the tube then begins to glide out slowly, and as its apex emerges, it may form short pseudopods and undergo amoeboid motion

at the anterior end (fig. 12). Since the diameter of the exit is less than that of the zoospores, the latter are always elongate as they emerge. When completely out, the swarmspore pauses a few seconds, rounds up, and begins to draw away. As this occurs, the long cilium becomes visible as a fine filament extending back into the exit tube (fig. 13). As one observes these successive stages, it becomes apparent that the cilium is developed before the zoospore escapes and that it may be pressed to the side of the exit tube wall by the successively emerging swarmspores. As a consequence, the free zoospore sometimes appears to be straining to get its cilium loose, as is illustrated in figure 13. After the cilium has been freed, the swarmspore may lie quiescent for a few seconds and then dart away suddenly.

In the meantime the second and succeeding spores have begun to glide up and out of the exit tube. This succession of spores occurs rather slowly, so that a sporangium 25 μ in diameter may require as much as 45 minutes to be emptied. So far no swarming of the zoospores in the sporangium prior to or during emission has been observed. This is another distinction between *C. sphaerocarpa* and *C. anguillulae*. After one to three swarmspores have escaped, those within the sporangium may glide slowly upon each other and make their way to the exit tube. The method of zoospore emission described above seems to me to be the normal and usual one, but, as in the case of *C. anguillulae*, unfavorable conditions, injury in mounting, premature deliquescence of the exit tube tip, etc., may lead to modifications. Accordingly, unless one studies a large number of dehiscing sporangia, one might readily be misled.

The resting spores of *C. sphaerocarpa* have been found on two occasions in internodes of *Chara coronata* and *Nitella flexilis*. They are predominantly spherical and slightly oval, 10–25 μ in diameter, but may sometimes be ellipsoidal, spindle-shaped, and elongate, as is shown in figures 17–20. Figure 17 shows three spores in tandem, while figure 20 illustrates a comparatively rare elongate type. They possess a relatively heavy brown wall, 1.5–2.5 μ thick, and their content is evenly granular in appearance without refractive globules, which are so characteristically present in the resting spores of most chytrids. While I have not yet observed their developmental stages, I nevertheless believe these spores are nothing more than ordinary sporangia which have encysted and developed thick walls. This is particularly suggestive in figure 18 of a spore with an exit tube whose lower portion has become thick-walled. Earlier, this spore was doubtless only a thin-walled sporangium which had attained the exit tube stage of development and then went into a resting condition and encysted. As far as I have been able to determine, the content of the sporangium does not contract before encysting as Buckley and Clapham (1929) have described for *C. anguillulae*, nor is sexuality involved in their development. No germination has yet been observed.

HOST RANGE AND PATHOGENICITY OF *C. SPHAEROCARPA*.—My observations to date show that *C. sphaerocarpa* is a saprophyte. It may often be found in partly green and dying algal cells, but experimental results have shown that it is not the cause of the pathological condition of the host. Extensive attempts have been made without positive results to infect normal healthy cells, and it is only after the host has been rendered pathological or killed that successful inoculation occurs. This species was first observed on September 21, 1936, in cooked and bleached cells of *Hydrodictyon reticulatum*, and since that time I have cultivated it successively in cooked cells of *Nitella flexilis*, *Chara coronata*, *Cladophora glomerata*, *Pithophora* sp., *Spirogyra crassa*, *Elodea canadense*, and root tips of *Zea Mays* and *Allium cepa*. I have also trapped it in nature by baiting various greenhouse tanks with these hosts. My measurements so far do not show any marked correlation between the size of the sporangia and that of the host cell, but the rhizomycelium appears to be more extensive in large cells like the internodes of *Chara* and *Nitella*. In the root tips of corn and onion a single thallus may occupy several cells.

FURTHER OBSERVATIONS ON *C. ANGUILLULAE*.—In 1934 the author described a saprophytic species of *Catenaria* occurring in a wide variety of killed and sterilized plant and animal tissues without identifying it with certainty. Additional studies of this species and cross inoculation experiments involving liver fluke, slug, and cockroach eggs have shown that it is undoubtedly *C. anguillulae* and revealed considerable data which were not particularly clear at that time. These data relate primarily to the reported fusion of motile isogametes and the method of zoospore emission and enable me to correct a misinterpretation made in my previous publication. What was then described and figured as probable fusion between two motile gametes now appears to have been based on observations of incomplete cleavage and the subsequent behavior of the abnormal segments after escaping from the sporangium. According to present observations text figures 1 to 7 of my earlier paper do not represent fusion stages between two gametes but the changes undergone by two zoospores which were incompletely delimited and failed to separate. Cleavage segments of unusual size and shape, such as are shown in figures 32 to 34, have been observed emerging from sporangia, and their subsequent behavior has been very striking and interesting. They may often remain actively motile for more than an hour with the cilia lashing back and forth in non-coordinate fashion, rolling and tumbling about, stretching into elongated strands, and undergoing other pronounced changes in shape from the intense and non-coordinate activity of the cilia. In the case of biciliate segments, such as were shown in my previous figures and the present figure 32, one may readily misinterpret their nature and significance unless the initial stages of origin are observed.

There is considerable difference of opinion in the literature as to the exact method of zoospore emission in *C. anguillulae*, and inasmuch as the nature of this process may often be of marked significance in species determination, I have made a detailed study of it for the purpose of comparison with *C. sphaerocarpa*. Sorokin (1876), E. J. Butler (1928), and Sparrow (1932) describe the swarmspore as emerging one by one and swimming directly away after a momentary pause at the mouth of the exit tube. Dangeard (1885), on the other hand, reports that they may emerge in two different ways: one by one in succession and swim away, or several together and lie quiescent in a globular mass surrounded by a mucous substance which dissolves in a few seconds and sets them free. J. B. Butler and Buckley (1928) confirm Dangeard as to the latter method. My observations relate to dehiscing sporangia in liver fluke ova and internodes of *Nitella* and *Chara* and confirm in most respects the findings of Butler and Buckley. As they have shown, the extreme tip of the exit tube appears as a more or less hemispherical region which is slightly opaque and greyish in appearance, as I have indicated by stippling in figure 25. This region may often be rather extensive (fig. 26), and as the process of deliquescence continues, it becomes convex underneath, swells, pushes up, and enlarges slightly at the outer periphery (fig. 28). With further swelling and deliquescence it becomes more hyaline, less visibly differentiated, and finally disappears entirely, leaving the tip open. In the final stages of deliquescence the zoospore in the sporangium and in the exit tube may begin to glide upon each other, but they are usually so closely packed together that any definite individual swarming is inhibited or impossible. This gliding movement may become so pronounced and synchronized as to involve the entire mass of spores within, and if the deliquescence of the tip is delayed for a few seconds, the whole mass may turn over and over in the sporangium. In a few cases no movement at all has been observed in the sporangium until some of the zoospores have escaped.

As the tip of the tube deliquesces, a comparatively small globular mass of fluid pushes out (fig. 29), and the initial spores rapidly emerge into it and cause it to expand. These initial spores may range from 5 to 50 in number and lie quiescent from 10 to 30 seconds imbedded in the more or less hyaline but microscopically visible globule of fluid. The latter soon dissolves in the water (fig. 30), its outline disappears, and very shortly the zoospores begin to swim away, as is shown in figure 31. As the pressure in the sporangium is thus apparently released, the zoospores within begin to swarm actively, so that the entire content becomes a violent, seething, and churning mass. At the same time, they continue to emerge singly or in pairs, depending on the diameter of the exit tube, pause momentarily at the orifice, and then swim directly away. It is thus obvious that if only these latter stages of zoospore emission are observed, one may be led to the conclusions of Sorokin (1876),

E. J. Butler (1928), and Sparrow (1932). From observations of a great number of sporangia I believe the process described above is the normal and usual manner of sporangium dehiscence and emission of the swarmspores.

If, however, conditions for emergence are not normal, mechanical obstructions occur, cleavage of the protoplasm in the exit tube is tardy, etc., numerous variations of the normal process may result, and this doubtless accounts for some of the discrepancies and differences of opinion in the literature as to the method of zoospore emission. In mounting the host tissues in which the sporangia occur, the long exit tubes may become twisted, bent, flattened, or depressed. As a consequence, it is difficult for the swarmspores to escape as soon as the tip deliquesces, and in numerous such instances observed, the spores have swarmed actively in the sporangium and swum directly away after emerging without forming a small globular mass at the mouth of the exit tube. Other less obvious factors of a similar nature may also delay emergence and cause aberrations of the normal process. In a few instances delayed cleavage of the protoplasm in the long exit tubes and the formation of large multiciliate segments which obstructed the orifice and prevented emergence of the spores completely have been observed.

GENERAL CONSIDERATIONS OF THE GENUS CATENARIA.

—As has already been pointed out in the beginning of this paper, there is considerable difference in opinion in the literature as to the size, shape, structure, and behavior of the zoospores of *C. anguillulae*. Villot (1874) and Sorokin (1876) described them as being spherical, 1.5–2 μ in diameter, and possessing a single conspicuous refractive globule. In 1885 and 1886 Dangeard reported what he believed to be the same species in *Anguilla* and *Nitella* and described the swarmspores as oval, approximately $3.4 \times 5.5 \mu$, with several small refractive granules instead of a single large one, and a habit of swimming which is more characteristic of *Oltipidiopsis*. Most subsequent workers, including J. B. Butler and Buckley (1928), E. J. Butler (1928), Buckley and Clapham (1929), J. B. Butler and Humphries (1932), and myself have confirmed Dangeard as to the shape, structure, and method of swimming of the zoospores but found that they are somewhat larger in size, $4\text{--}5 \mu \times 6\text{--}7.5 \mu$. Sparrow (1932), however, has described the swarmspores of a species which he found in a rotifer as being spherical, 2 μ in diameter, with one and often two refractive globules, thus confirming Villot and Sorokin as to size. With the view of presenting these differences more concretely, I have copied to scale and brought together in figures 21, 22, 23, and 24 the zoospores of *C. anguillulae* as they have been figured by Villot, Sorokin, myself, Butler and Buckley, and E. J. Butler, respectively. The differences here shown are sufficiently striking and significant to raise the question as to whether more than one species is included in *C. anguillulae*. It is possible that the early measurements by Villot and Sorokin are incor-

rect, but since they have recently been confirmed by Sparrow, this seems less probable. In the interests of clarity it may perhaps be worth while to limit the binomial *C. anguillulae* to Villot, Sorokin, and Sparrow's fungus and designate the species discovered by Dangeard and subsequently studied by Constantineanu, E. J. Butler, J. B. Butler and Buckley, Buckley and Clapham, J. B. Butler and Humphries, and myself as *C. dangeardii*. It is to be noted in this connection, however, that the sporangia and other portions of the thallus figured by Villot and Sorokin are strikingly similar to those of Dangeard's fungus; and while the size and shape of sporangia are not, in my opinion, always of significant diagnostic value, I none the less believe that in this respect there is some evidence for identity of the two forms. Accordingly until further evidence for separating the two are found, I believe it prudent to leave *C. anguillulae* as it is now treated in the literature.

The question naturally arises in this connection as to whether *C. sphaerocarpa* may be identical with Villot and Sorokin's fungus, since its zoospores are also spherical and possess a single conspicuous refractive globule. While this is not altogether improbable, the latter fungus is so imperfectly described it is difficult to make comparisons. The outstanding differences are the size of the zoospores and the shape of the zoosporangia. That *C. sphaerocarpa* is different from Dangeard's fungus there is no doubt.

Catenaria pygmaea Serbinow is a very questionable species and should doubtless be removed from the genus. According to Serbinow's (1907) figures, the thallus is monocentric with a coarse rhizoidal system which penetrates several host cells and is delimited from the sporangium by a cross wall, while the small, 1.5 μ , spherical zoospores with clear refractive globules escape in a globular mass and lie quiescent at the mouth of the exit papilla before swimming away. The large sporangia may become depressed and cylindrical in the narrow algal filaments in the same manner as I have described (1937) for *Endochytrium operculatum*. *Catenaria pygmaea* thus has many structural characters in common with *Endochytrium*,

and as I have indicated elsewhere (1937), it probably belongs in this genus, although Minden (1911) regards it as a member of *Eutophlyctis*.

In some respects the thallus of *Catenaria* resembles that of *Mitochytridium*, particularly when the latter becomes polycentric and develops resting spores. Couch (1935) is accordingly of the opinion that they may show some relationships should the former be found to have cellulose walls. I have made repeated tests of both *C. anguillulae* and *C. sphaerocarpa* with chloro-iodide of zinc without finding any positive cellulose reaction.

SUMMARY

Catenaria sphaerocarpa n. sp. is a saprophyte in dying and dead algal cells and can be grown successfully in cooked root tips of *Zea Mays* and *Allium cepa*. All attempts to infect healthy normal cells have so far failed.

This species is distinguished from *C. anguillulae* primarily by large spherical zoospores with a single, conspicuous refractive globule and a characteristic type of swimming, brown resting spores, and predominantly spherical zoosporangia. The resting spores appear to be formed in the same manner as the zoosporangia by encystment and the development of a thick wall.

In a further study of *C. anguillulae* it has been found that biciliate and multiciliate abnormal cleavage segments may be formed and emitted with the normal zoospores. The appearance and subsequent behavior of the biciliate segments particularly may often give the impression that they are zygotic and have been formed by fusion of two gametes.

The zoospores of *C. anguillulae* as described and figured in the literature vary markedly in size, shape, structure, and behavior, and there is accordingly a strong possibility that two species are included under this binomial.

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A NEW FERN FROM THE JOHNS VALLEY SHALE OF OKLAHOMA¹

Charles B. Read

IN JANUARY, 1937, Mr. T. A. Hendricks of the United States Geological Survey submitted to the writer a silicified specimen of a fossil fern stem with several zygopterid phyllophores or "petioles" attached. The specimen was collected in the Ouachita Mountains of Oklahoma from an outcrop of Johns Valley shale [early Pennsylvanian] in sec. 6, T. 1 S., R. 13 E.

The following statement regarding the occurrence has been prepared by Mr. Hendricks: "The Johns Valley shale contains boulders derived from older formations. Boulders derived from formations as old as Cambrian and as young as early Pennsylvanian have been identified. The specimen described herein may be native to the Johns Valley shale or may be an exotic boulder derived from some older formation."

In the opinion of the author of this paper it is probable that the fossil here described is indigenous to the Johns Valley shale and is not a boulder.

At the moment it is impossible to go far into the details of morphology of this fossil due to other very urgent duties. There are, however, some features of the specimen which deserve immediate mention and description. Therefore, the following brief statement has been prepared.

DESCRIPTION.—*Ankyropteris hendricksi* n. sp.—**General features.**—The fossil is a fragment approximately six inches in length and is slightly flattened so that in transverse section the mass is irregularly oval. The preservation is through the agency of silica, the color of the specimen on broken or polished surfaces being a light, mottled brown.

When viewed megascopically on transverse surfaces it is seen that there are present numerous, large organs which may be identified as zygopterid "petioles" or phyllophores. To one side of the block is a somewhat fleshy appearing stem which bears a relatively small stele from which numerous, small vascular strands emerge in a complex and rather close spiral. It is clear from the constant orientation as well as the actual attachment in a few instances that the phyllophores are related to the stem and are in a continuation of the spiral arrangement of the vascular strands lying in the peripheral portions of the stem.

The stem and the several phyllophores are embedded in a mass or sort of ramentum of small,

densely packed roots. It is notable that these roots freely traverse the tissues of the phyllophores farthest removed from the stem, a circumstance clearly indicating the persistence of the leaf-bearing organs.

Stem.—The stem is about 30 mm. in diameter, of which nearly 8 mm. are occupied by the cauline stele. In transverse section it is apparent that this stele varies from octagonal to heptagonal. It is inclined, however, to be irregularly so, due to the varying development or prominence of the angles. In the center is a small area approximately 1 mm. in diameter which is occupied by clear silica. Immediately surrounding this is a narrow zone of rather crushed tracheids and parenchyma. Adjacent is the great bulk of typical primary xylem, polygonal in form. The protoxylem is mesarch and is situated near the exterior of the column in the lobate areas formed by the angles. The tracheids of the primary xylem are large and rather angular. The pitting is scalariform. Prominent parenchymatous raylike bands of tissue extend outward from the pith into the lobes. Surrounding the xylem is a narrow zone of indifferent preservation which represents the outer stellar tissues.

The outer tissues of the stem, with the exception of the decurrent leaf traces, are entirely parenchymatous. A mass of filamentous, hairlike emergences covers the epidermis.

The phyllotaxis appears to be normally about 2/7. The stele of the stem, as will be seen from the brief account given above, is a modification of the familiar stellate type characteristic of the large proportion of Zygopterideae and in particular of the species of *Ankyropteris* such as *A. grayi* (Williamson) Bertrand (Bertrand, 1909, 1912; Scott, 1912) and *A. corrugata* (Williamson) Bertrand (Bertrand, 1909, 1912; Holden, 1930).

The phyllophore trace.—Phyllophore is a term proposed by Bertrand for the so-called primary rachis of the zygopterid ferns. This organ differs from the typical petiole or rachis in its symmetry which is axial as opposed to the bilateral symmetry and dorsiventrality of the typical petioles. This is shown by the pinnae traces which may be two or four ranked on the rachis and are oriented at right angles to the primary rachis. It will be recalled that ordinarily the principal planes of symmetry of the rachis and pinnae are parallel rather than at right angles. Bertrand regards the primary rachis of this former type as characteristic of the Zygopterideae. Since the organ in question does differ from the typical primary rachis

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the writer prefers to use the special term which has been proposed for it (Bertrand, 1903).

The phyllophore trace in its origin is similar to other known species of *Ankyropteris* (Bertrand, 1900; Holden, 1930; Scott, 1912, 1920). The trace, as it pinches off the lobes of the xylem, is triangular, proto-stelic, and endarch. As the bundle emerges, it next becomes oval and then crescentic, the concavity being directed outward. Gradually the protoxylem, which is originally one, divides so that a group appears at either end of the flattened bundle. Then in the base of the phyllophore the strand assumes the "H" shape. The details of this series of stages have not been worked out, but it is clear that there is a conformity with other species of the genus.

The phyllophore.—The phyllophores are large, fleshy-appearing organs ranging up to about 25 mm. in diameter. The center of each is occupied by an H-shaped trace, the free ends of the H being pulled together slightly. The protoxylem is situated along the outer sides of the "H" and is rather evenly distributed save for a narrow space opposite the ends of the central bar. Surrounding the trace is a zone of fine-celled parenchyma probably referable to the phloem. The stele is sheathed in a dense, small-celled parenchyma of considerable extent so that the phyllophore suggests in its general appearance a fleshy or succulent organ. The epidermis is, as in the case of the stem, densely clothed with small hairlike emergences.

As regards pinnae and aphanlebiae traces, these are very interesting in the specimen under consideration. The pinnae are indistinguishable from the aphanlebiae except for their position. It will be noted upon examination of the general view of the transverse section of the specimen that there appear to be three rows of appendages, each carrying a small vascular bundle, emerging from each side of the phyllophore. The vascular strands are small, flattened, yet annular structures. They are similar, in fact identical in so far as the preservation permits observation. Their general behavior and their appearance when seen partially or wholly detached and adjacent to the phyllophores is very suggestive of the aphanlebiae in *Ankyropteris corrugata* described by Holden (1930). The writer's interpretation of the situation is that the central trace is a true pinna trace, while the lateral ones are those of aphanlebiae. The lack of differentiation may be due to primitiveness or to reduction. At present the former appears to be more reasonable, but a discussion of such a highly theoretical point is outside the scope of this paper (Brown, 1935). A very similar situation exists in *A. corrugata*, where, according to Holden, it is very unlikely that any differentiated pinnae exist. His statement that "The probabilities are that the petioles were totally devoid of flattened pinnules and that, apart from bristle-like hairs and aphanlebiae, they were quite naked" seems to apply equally well to *A. hendricksi*.

Discussion.—In the short account just given it has been shown that *Ankyropteris hendricksi* is char-

acterized by a stem with a heptagonal stellate stele with phyllophores arranged in a close spiral, by phyllophores exhibiting the characteristic H-shaped or eta outline and showing little if any differentiation between aphanlebiae and pinnae in so far as the lateral organs of the phyllophores are concerned. Phyllophores and stem were bound together by adventitious roots which were diarch and by abundant hairlike protuberances from the epidermis. It is likely that the whole structure was upright and in habit similar to some of the low tree ferns of the present day. The "petioles" appear to have been very persistent. This is indicated by the frequent presence of adventitious roots cutting the phyllophores. Such a condition suggests that the growing point of the stem was some distance above and that these functionless phyllophores were below any apical crown of functioning phyllophores. The presence at this level of phyllophores obviously functionless and partly broken down and penetrated by roots is indicative of the lack of development of any abscission layer and of the persistence, probably of broken stubs, of these phyllophores below the main crown of leaves.

Of the seven known species of *Ankyropteris*—namely, *A. bibractensis* Bertrand, *A. bronngiarti* Bertrand, *A. corrugata* Bertrand, *A. grayi* Bertrand, *A. scandens* Bertrand, *A. westphaliensis* Bertrand, and *A. williamsoni* Bertrand—the specimen which has been described above as *Ankyropteris hendricksi* probably resembles most closely *A. corrugata*. In the outline and general structure of the cauline stele and in the aphanlebioid condition described above there are features of marked similarity. However, in the distichous arrangement of the phyllophores as well as in the form of the phyllophore bundle there are notable differences. There is a somewhat superficial resemblance between the phyllophore bundle form in *A. grayi* and *A. hendricksi*. Clearly, though, the occurrence of differentiated pinnae on *A. grayi* sets that form apart. The stem structure is likewise different in detail although similar in general aspect.

At least one anatomical feature of this fern suggests that it is to be regarded as a primitive member of the genus. The undifferentiated pinnae traces which are indistinguishable from the aphanlebiae establish the form as far more primitive than the other species with the exception of *A. corrugata* where a similar situation exists (Brown, 1935; Holden, 1930).

SUMMARY

A new fern, *Ankyropteris hendricksi*, is described from silicified material of phyllophores, stem, and roots. It was collected from early Pennsylvanian sediments in the Ouachita Mountains of Oklahoma, although it may be an exotic boulder derived from pre-Pennsylvanian strata. The fern is characterized by (1) a small octagonal to heptagonal stem stele, siphonostelic, and similar to, yet differing in detail from *A. grayi*; (2) large, fleshy-appearing phyllophores marked by the familiar H-shaped vascular strands; (3) a lack of differentiation of pinnae. Aph-

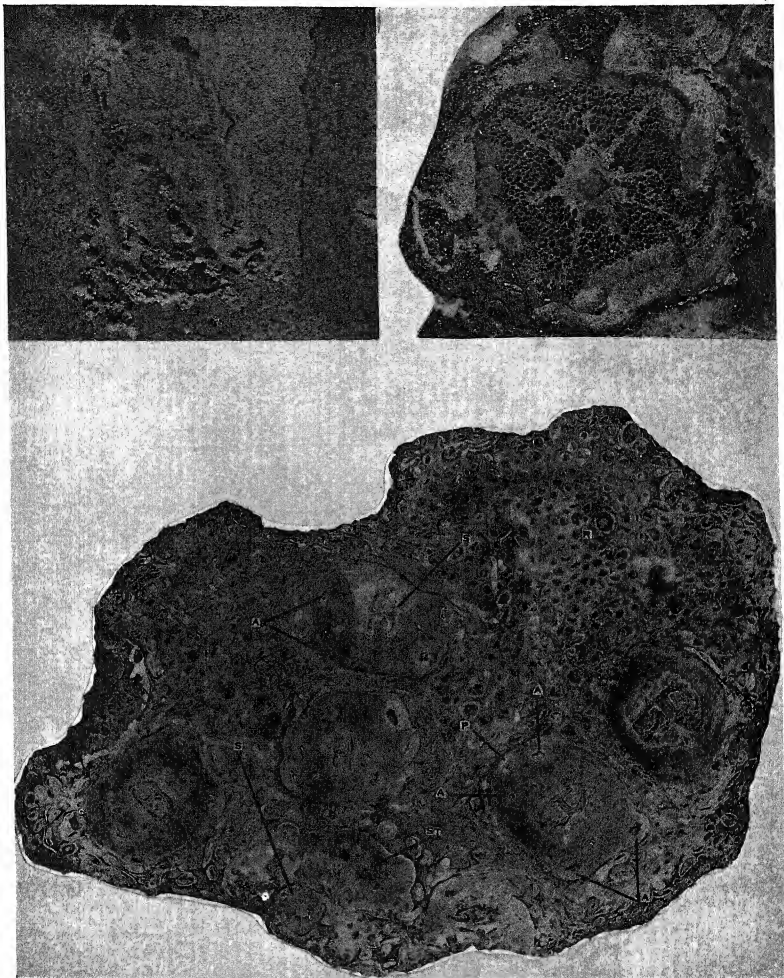


Fig. 1-3.—Fig. 1 (upper left). Photograph of the H-shaped vascular bundle of the phyllophore of *Ankyropteris hendricksi*. $\times 9\frac{1}{2}$ —Fig. 2 (upper right). Photograph of the stele and adjacent tissues of the stem. Note the slightly stellate outline of the vascular column and the mesarch position of the protoxylems. $\times 9\frac{1}{2}$ —Fig. 3 (below). Photograph of a transverse cut of the specimen of *Ankyropteris hendricksi* showing the stem, phyllophores, and the investing mass of roots. *st*, stem; *s*, stele of the stem; *s'*, stele of the phyllophore; *a*, aplebia; *p*, pinna base. $\times 1\frac{1}{4}$. (Note: All illustrations are of polished surfaces taken with reflected light.)

leaves which are given off in two rows on either side of the pinnae are anatomically indistinguishable from the rudimentary pinnae. (4) Stem and phyllophores are clothed by a dense mat of epidermal hairs, and the mass is bound together by abundant adventitious roots which form a sort of ramentum. Thus a structure approaching that of some modern low tree

ferns is developed. (5) The lack of differentiation of pinnae is taken to indicate that this species is a rather primitive member of the genus.

GEOLOGICAL SURVEY,

U. S. DEPARTMENT OF THE INTERIOR,
WASHINGTON, D. C.

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HETEROTHALLISM AND VARIABILITY IN *VENTURIA INAEQUALIS*¹

G. W. Keitt and D. H. Palmer

KNOWLEDGE of the occurrence, nature, and cause of variability of the pathogen is basic to understanding of the development and the control of infectious disease. Recent advances in the knowledge of reproduction and inheritance in the fungi (Bensaude, 1918; Shear and Dodge, 1927; Dodge, 1927; Knipf, 1928; Buller, 1931; Craigie, 1931; Drayton, 1934, and others) have greatly encouraged and facilitated genetic studies of pathogenic fungi in relation to phytopathological problems.

Venturia inaequalis (Cke.) Wint. is the inciting cause of apple (*Malus sylvestris* Mill.) scab, the most widespread and destructive disease of deciduous orchard fruits. The possibility that this species contains biotypes that differ in such important characters as pathogenicity and reaction to fungicides has long been suggested. However, with the exception of work on responses to environmental conditions, variability in this classical fungus received little critical study prior to the investigations of Wiesmann (1931) and Palmer (1932, 1934), who showed independently that monoclinal isolates differed substantially from one another in morphology, pathogenicity, and various physiological responses. Palmer (1934) states that his findings . . . "indicate that *V. inaequalis* is not a homogeneous species or one made up of a few well defined forms with definite cultural and pathogenic reactions but is one made up of many strains that differ in various degrees in their morphologic and physiologic characters." The essential findings of Wiesmann and Palmer were confirmed and extended by the investigations of Rudloff (1934), Schmidt (1935), and Kärthe (1935). Herbst, Rudloff, and Schmidt (1937) state that extensive efforts to

produce ripe asci of *V. inaequalis* in artificial culture yielded only negative results. However, they report finding mature ascospores in tube cultures of certain races of *Venturia ditricha* (Fr.) Karst. They conclude that these races are homothallic, but suggest the possibility that heterothallic races of this species may also occur. They state that Killian's cytological studies on sexuality of *V. inaequalis* and the results of unpublished work in which they secured an abundance of morphologically differentiated types by monosporic isolations from naturally occurring perithecia suggest heterothallism in this species. They conclude that, although validly exact proof is lacking, it may be assumed that the morphotypes of *V. inaequalis* are hereditary races in which combinations occur. Schmidt (1935) expresses the view that valid proof that these morphologic differences depend on differences in genetic constitution can be obtained only by crossing experiments.

The development of the ascocarp of *V. inaequalis* has been critically studied by Killian (1917) and Frey (1924), whose findings are in general agreement, though they differ in certain details. Both authors review extensive literature dealing with sexuality in the Ascomycetes.

According to Killian, the perithecium is initiated by a helical growth at the apex of a hypha that is indistinguishable from vegetative hyphae. A coiled archicarp, or ascogonium, surrounded by the initial of a perithecial wall and provided with a well differentiated trichogyne, develops from this fundament. The apex of another hypha of the vegetative type develops into a well differentiated multinucleate antheridium, which comes in contact with the trichogyne. A pore is formed in the walls, and the nuclei of the antheridium pass through the trichogyne into the ascogonial coil, which at this stage typically con-

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sists of about seven cells, each containing two to several nuclei. The septa of the ascogonial coil are dissolved and the antheridial nuclei mingle with those of the ascogonium. A tendency to pairing was observed in both the antheridial and the ascogonial nuclei before they mingled, and also in the mingled nuclei. Since the mingled antheridial and ascogonial nuclei were not differentiated from each other by the cytological methods employed, conclusions were not reached as to whether or not each pair was comprised of one nucleus from each of these organs. The middle cells of the archicarp give rise to multinucleate cells from which arises a system of ascogenous hyphae consisting of cells that are typically binucleate. Crozier formation occurs, the penultimate cell develops into the ascus, and its two nuclei fuse to form the primary ascus nucleus. The usual three divisions then follow, and eight ascospores are delimited. The mature ascospore, which consists of two uninucleate cells, may germinate and give rise to a vegetative mycelium composed of uninucleate cells.

Kilian states that it is seldom possible to trace the origin of the antheridial hypha, but that in one case it was definitely traced to the plectenchyma of the Fusicladial stage. He concludes that, since the primary hyphae were originally widely separated, often growing from different surfaces of the leaf, the fungus is heterothallic according to Blakeslee's terminology. Kniep (1928), however, states that this conclusion is unsubstantiated so long as it is not demonstrated that the respective sexual organs always arise from different mycelia that are derived from different ascospores.

The available cytological evidence accords perfectly with an hypothesis that the ascospores of *V. inaequalis* are formed in consequence of a sexual process, which provides the mechanism for combinations. However, since various types of so-called reduced fertilization have been reported in the Ascomycetes, an element of doubt must attach to the interpretation of fertilization in this fungus and its relation to heritable variation, until it is definitely known whether both antheridium and ascogonium contribute hereditary materials to the ascospores and whether functional and compatible antheridia and ascogonia are borne on the same thallus.

The purpose of the present paper is to report the earlier results of a genetic study designed to supplement the cytological and cultural evidence relating to sexuality and inheritance in *V. inaequalis*. A preliminary report (Keitt and Palmiter, 1937a) containing experimental proof that this fungus is heterothallic, in the sense that it is hermaphroditic, self-sterile, intra-group sterile, and inter-group fertile, was given before the Mycological Society of America, December 30, 1936.

ISOLATION OF THE EIGHT SPORES FROM AN ASCUS.—In July, 1935, the eight spores of an ascus were isolated by the following method from a Dudley apple leaf that had been collected at Sturgeon Bay in May and held air-dry in a refrigerator at 4°C. The leaf

was placed in tap water for a few minutes. Under the dissecting microscope a suitable perithecium was removed with a needle, placed in a drop of sterile water on a sterile glass slide, and crushed. The slide bearing the crushed perithecium was examined under a dissecting microscope provided with a condenser, artificial illumination, and lenses that permitted satisfactory observation of the individual ascospores with ample room for the necessary manipulations. Several drops of sterile distilled water were placed separately on a sterile slide. A well developed ascus bearing eight olivaceous spores was drawn by capillarity into a sterile glass tube just large enough to permit its entrance. The ascus was then blown out of the tube into one of the drops of sterile water, with precautions against contamination, and observed to make sure that no extraneous spores accompanied it. This process was repeated twice. In the third drop the ascus wall was broken and the spores were separated by means of a fine closed capillary glass tube, slightly curved at the end. A few drops of melted malt agar, suitably cooled, were then mixed with the drop of water and allowed to solidify on the slide, which was placed in a sterile Petri dish lined with moist filter paper. Soon after the spores germinated, a small disc of the medium about each was cut by Keitt's (1915) method and transferred to a malt agar plate for further development and observation. Transfers were later made to tubes. Stock cultures of the eight isolates were maintained throughout these studies on malt agar or potato dextrose agar slants at 8°C.

The method of isolation just described is far from satisfactory. Usually one or more spores of a set fail to germinate, and the serial order in which they occur in the ascus is not recorded. In subsequent work this technique has been superseded by a much more efficient one, which will be reported in a later paper.

BREEDING THE FUNGUS IN APPLE LEAVES.—In order to gain evidence as to whether the fungus is heterothallic or homothallic, it was sought to study the eight isolates singly and in all possible pairings under conditions favorable for production of perithecia. Since methods for production of the ascleigerous stage at will in vitro were not known, it was decided to culture the fungus on apple leaves, simulating natural conditions for perithecial development.

Materials and methods.—Two-year-old Fameuse apple trees were obtained from a nursery in the spring. The tops were cut back to about two feet and the roots trimmed to fit into 8-inch pots. The trees were then repacked and kept dormant in cold storage until early September, when they were removed and potted. After ten days in a basement they were placed in the greenhouse, where two shoots were allowed to grow on each tree. After about six leaves per shoot had developed, the trees were inoculated with conidia obtained from cheesecloth "wicks" adherent to the inner wall of 12-ounce bottles containing 20 cc. each of 2.5 per cent malt extract (Trommer's) solution. The bottles were planted by lightly smearing the cheesecloth surface with mycelium from

agar cultures. The cultures were incubated at 16°C. for about ten days before they were ready for use. Before removing the spores, the nutrient solution was decanted and the bottle partly filled with distilled water. Upon shaking, the conidia were washed from the "wick," and the spore suspension was placed in an atomizer. The upper and lower surfaces of the leaves were sprayed with the spore suspension.

Each of the eight isolates was used alone and in all possible pairings. One tree was inoculated with all eight isolates. Three control trees were left uninoculated but otherwise treated as the inoculated trees.

In the experiment of 1935-1936 the trees were inoculated October 11, kept in the moist chamber (Keitt and Jones, 1926, fig. 8) at 16 to 18°C. for 48 hours, and returned to the greenhouse, where the temperature was kept at about 16° and the relative humidity at about 60 to 85 per cent. Care was taken throughout the experiment to keep the trees as well separated as feasible in order to avoid accidental transmission of the fungus. All the inoculated trees showed abundant infection except those inoculated with isolate 4 alone, 1 alone, and 4 + 7. In each of these cases, in which the trees were not vigorous, only a few lesions appeared. On November 8, the leaves showing abundant infection were picked and placed with the dorsal surface up in cloth net bags (Keitt and Palmiter, 1937b, fig. 1), which were laid on sod out of doors to overwinter. The inoculation experiment was repeated on younger leaves of the same trees on October 25 or 28. These leaves were picked November 29 and kept in a cold greenhouse until they could be placed on the ground on December 9.

The uninoculated trees showed no evidence of infection, but the leaves were picked and put down to overwinter as controls.

The bags were brought into the laboratory May 13 and the leaves examined microscopically for mature perithecia of *V. inaequalis*. Production of ascospores was the criterion of maturity of perithecia. Due to the delicacy of the foliage produced in the greenhouse and to weather conditions in the spring, many of the experimental leaves disintegrated before the ascocarps were mature. Some samples were entirely missing, and of others only a few fragmented leaves remained.

One infected leaf from each inoculated tree was overwintered on the outside ledge of a north window. The leaves were fastened together in small bundles, each leaf being separated from the next by a piece of paper towel. The bundles were moistened from time to time, and in the spring they were placed in controlled temperature chambers for maturation of the perithecia. The number of mature perithecia per leaf was determined by microscopic examination.

The experiment was repeated in 1936-1937. The trees, however, were inoculated but once, and all the leaves were overwintered out of doors. There was much less disintegration of leaves than in the first experiment.

Results in 1935-1936.—The results from the leaves overwintered in cloth net bags in 1935-1936 are shown in table 1. Although many of the samples were miss-

TABLE 1. Number of perithecia of *V. inaequalis* produced per square inch in apple leaves in relation to pairings of eight monoascospore isolates from a single ascus, 1935-1936. (The leaves were overwintered out of doors. Disintegration occasioned loss of the samples for which data are lacking.)

		Isolate number:							
		1	2	6	8	3	4	5	7
Isolate number:	1	0	...	48	19	29	...
	2		0	..	0	41	69	123	58
	6			0	...	130	...	100	6
	8				51
	3					0	0
	4					
	5						
	7								0

ing, the data show that the eight isolates can be divided into two groups (hereinafter called sterility² groups) of four each (1, 2, 6, 8 and 3, 4, 5, 7). When a single isolate from either group was used alone, no mature perithecia were produced. Inoculations made with any two isolates from the same group also failed to lead to the production of mature perithecia. In each available sample in which an isolate of either group was paired with one of the other group, mature perithecia were produced. No perithecia of *V. inaequalis* were borne in the uninoculated leaves, whereas the leaves inoculated with all eight isolates produced mature perithecia abundantly.

The results from the leaves overwintered between paper towels are shown in table 2. All the leaves being in good condition, a complete record was obtained. With three exceptions, the results obtained conform with the evidence shown in table 1. In each of these three cases (isolates 2 + 6, 2 + 8, and 6 + 8), mature perithecia (1, 2, and 48, respectively) were found in a localized area, whereas none was expected. It seems probable that they were the result of contamination.

Portions of leaves were cleared according to the method of Peace (1910). In cases in which isolates

² In this paper isolates or pairings are referred to as sterile if no ascospores were produced under the most favorable environmental conditions provided in these studies. The criterion of fertility was the production of ascospores. The writers regard production of viable ascospores as a preferable criterion of fertility, but exigencies of the experimental program prevented germination tests from all fertile pairings. In all such tests made, however, the ascospores were viable. The terms sterility and fertility, as used herein, have no reference to the production of conidia.

For convenience of statement, isolates capable of cross-fertility when mated with one another are referred to as compatible; those incapable of cross-fertility when mated with one another, as incompatible.

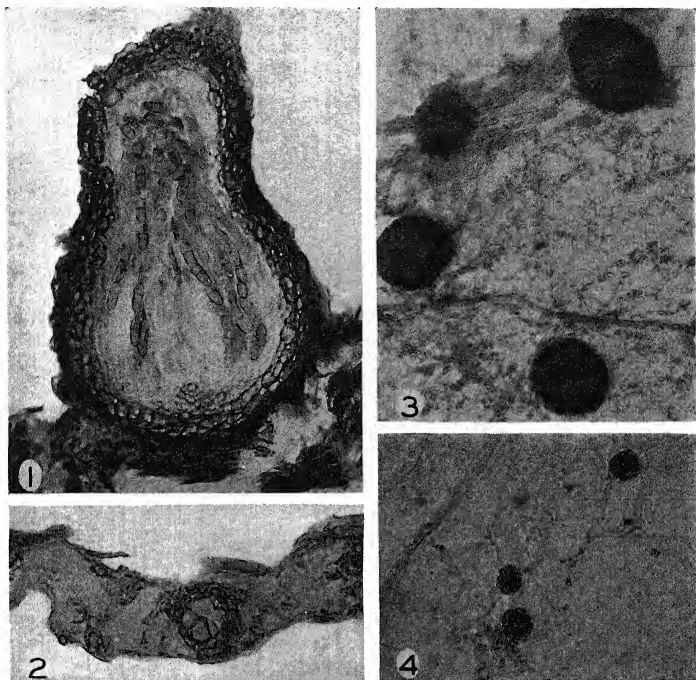


Fig. 1-4.—Development of ascocarps of *V. inaequalis* in relation to the pairing of isolates.—Fig. 1. Section of an overwintered apple leaf that had been inoculated with two isolates (1 and 3) of different sterility groups, showing a normal perithecium ($\times 350$).—Fig. 2. Corresponding section of a leaf inoculated with isolate 3 alone, showing the remains of an ascocarp initial ($\times 350$).—Fig. 3. Cleared leaf inoculated with two isolates (2 and 7) of different sterility groups, showing mature perithecia ($\times 100$).—Fig. 4. Similar leaf inoculated with isolates (3 and 7) of the same sterility group, showing remains of ascocarp initials ($\times 100$).

of different sterility groups had been used for the inoculation, normal mature perithecia were observed (fig. 1, 3). In those in which only one isolate was used for the inoculum, or two isolates of the same sterility group, numerous ascocarp initials were found (fig. 2, 4), but they were sterile and usually attained less than one-half the diameter of normal mature perithecia.

Results in 1936-1937.—The results obtained in 1936-1937, which are shown in table 3, confirm those of the previous experiment (table 1, 2). Perithecia were produced in all the expectedly fertile pairings from which material was available, only two samples in this category having disintegrated so badly as to preclude examination. No perithecia were found in any other samples. It is noteworthy that no peri-

thecia were found in the pairings 2+6, 2+8, and 6+8, which had given apparently aberrant results in the earlier experiment in which one leaf from each inoculation had been overwintered between paper towels on the window ledge (table 2).

BREEDING THE FUNGUS IN VITRO.—The only instance known to the present writers in which ascocarps of *V. inaequalis* have hitherto been brought to maturity in vitro was reported by Jones (1914), who found perithecia containing mature ascospores in tube cultures that had been left for some months in a refrigerator. Jones was able to repeat his experiment, using the original cultures and cultures isolated from the ascospores produced in vitro. Both apple leaf agar and oatmeal agar were used successfully, the cultures being incubated at 10°C . for about five or

TABLE 2. Number of perithecia of *V. inaequalis* produced per apple leaf in relation to pairings of eight monoaecospore isolates from a single ascus, 1935-1936. (One leaf of each category was overwintered between paper towels on the outer ledge of a north window.)

Isolate number:	Isolate number:							
	1	2	6	8	3	4	5	7
1	0	0	0	0	142	57	870	106
2		0	1	2 ^a	74	394	56	122
6			0	48 ^a	103	112	160	172
8				0	10	197	159	41
3					0	0	0	0
4						0	0	0
5							0	0
7								0

^a These perithecia occurred in a localized area. See text.

six months. It appears that Jones' success is attributable chiefly to the method by which he isolated his cultures. Perithecia were permitted to discharge ascospores upon the surface of an agar plate. A portion of the agar bearing spores free from contaminants was transferred to a tube. He thus worked with polyascospore cultures.

Aderhold (1896), Rudloff (1934), Schmidt (1935), Herbst, Rudloff, and Schmidt (1937), and others have reported only negative results from attempts to produce ascospores in vitro, though perithecial initials were often observed. While the production of the ascleurogous stage in vitro was not a special objective of the present authors prior to the investigation herein reported, examination of many cultures incubated at temperatures near 10°C. on oatmeal agar and other agar media revealed no ascospores, though perithecial initials were found in abundance. Most of the cultures used were monosporic, but mixed cultures containing several isolates were sometimes employed. Proof of the heterothallic nature of the

TABLE 3. Number of perithecia of *V. inaequalis* produced per sample^a of apple leaves in relation to pairings of eight monoaecospore isolates from a single ascus, 1930-1937. (The leaves were overwintered out of doors.)

Isolate number:	Isolate number:							
	1	2	6	8	3	4	5	7
1	0	0	0	0	185	308	38	52
2		0	0	0	266	83	..	102
6			0	0	428	248	13	118
8				0	324	97	..	92
3					0	0
4						0	..	0
5						
7								0

^a The leaves from each inoculation comprised a sample. The perithecia were found on 2 to 6 leaves per sample.

fungus and the availability of isolates of known compatibility encouraged an attempt to breed the organism in vitro.

Methods and materials.—The eight monoaecospore isolates from an ascus were grown singly and in all possible pairings on cheesecloth "wicks" adherent to the sides of bottles containing 20 cc. each of 2.5 per cent malt extract solution, as described for production of conidia. After planting, the bottles were kept at 16°C. for 17 days until the mycelium had well covered the cheesecloth. They were then placed at 8° to check vegetative growth. Ten days later the malt extract solution was decanted, and sterilized 20 cc. portions of a weak decoction of dead apple leaves were substituted. When the cultures had been at 8° for 45 days, the temperature was raised to 12°, and after 39 days more it was raised to 16° (Wilson, 1928). An examination 24 days later revealed very little advancement, so the temperature was dropped to 12° for 30 days and then raised to 16° until the ascospores were mature. The gross development of ascocarps was followed by examination through the walls of the bottles by means of a dissecting microscope.

Results.—Perithecial initials were observed in some of the cultures 41 days after the experiment was started, or 15 days after the leaf decoction was substituted for the malt extract solution. Twenty days later about half the cultures showed abundant perithecial initials and most of the others a few. When examined again two months later, the perithecia were distinctly larger in all cultures of compatible pairs than in those of incompatible pairs or single isolates.

In a final microscopic examination about six months after the beginning of the experiment, no ascospores were found in cultures of single isolates or incompatible pairs. Only the following cultures of compatible pairs yielded perithecia in which ascospores were observed: 8 × 3, 8 × 4, 8 × 5, and 8 × 7. It is noteworthy that isolate 8 was cross-fertile when paired with any of the 4 compatible isolates. The failure of the other expectedly fertile pairings to yield ascospores is not explained. It would appear that the conditions were marginal for production of mature perithecia and that isolate 8 was more effective than the others in contributing to sexual reproduction under these circumstances.

DIFFERENCES IN PATHOGENICITY OF THE EIGHT ISOLATES.—In an earlier study, one of the writers (Palmiter, 1934) demonstrated that monocoenidial isolates of *V. inaequalis* may differ in their ability to infect certain apple varieties. Among the varieties that were infected by some of the isolates and not by others were Yellow Transparent, McIntosh, Missouri Pippin, and Hubbardston Nonsuch. Consequently, these were selected for tests for differences in pathogenicity of the eight monoaecospore isolates used in the present study. Fameuse, which was infected by all isolates tried in the earlier work, was included for comparison.

Methods and materials.—The experimental trees were handled and inoculated as described earlier in this paper. The conidia were produced on cheese-cloth "wicks" as already described, using a 2.5 per cent malt extract solution as the culture medium. One tree of each variety was inoculated with a given isolate, placed in the moist chamber at 18°C. for 48 hours, and returned to a greenhouse held at about

TABLE 4. Differences in pathogenicity of eight monosporic isolates from an ascus of *V. inaequalis* on five apple varieties inoculated in the greenhouse.

Variety	Results ^a from stated isolates:							
	1	2	3	4	5	6	7	8
Yellow								
Transparent	++	0	0	++	0	0	..	++
McIntosh ...	++	0	0	++	0	0	++	++
Missouri								
Pippin	+	++	++	+	++	++	+	+
Hubbardston								
Nonsuch ...	++	++	++	++	..	++	++	++
Fameuse	++	++	++	++	++	++	++	++

^a ++ = Abundantly sporulating lesions. + = Sparsely sporulating lesions. 0 = Lesions nonsporulating or no lesions.

16°. The isolates that did not cause infection on certain varieties in the first inoculation were used in a second trial on the same and different trees of those varieties.

Results.—The results of the studies on pathogenicity are summarized in table 4. Three of the five apple varieties used were infected by each of the eight isolates, but there were differences in the quantity of conidia produced by the different isolates on Missouri Pippin, 2, 3, 5, and 6 sporulating more

abundantly on this variety than 1, 4, 7, and 8. Yellow Transparent and McIntosh were infected by isolates 1, 4, 7 and 8 but not by the other four isolates, save for flecking without production of conidia. Two isolates of each sterility group comprise each group of four of like pathogenicity.

Differences in cultural characters of the eight isolates.—Only a limited examination has been made of the cultural characters of the eight monosporic isolates under discussion, as studies beginning with freshly isolated sets of cultures from ascospores of known serial arrangement in the ascus are preferred for the purposes of this investigation. Such studies are in progress, and will be reported in a later paper.

Methods and materials.—Mycelial transfers from the eight isolates were used to plant Petri plates containing 25 cc. each of an agar medium, one colony being produced per plate. Malt extract agar (Trommer's malt extract, 2.5 per cent; agar, 1.7 per cent) was used in one series and potato dextrose agar in another. After incubation at 16°C. for 24 days, the average diameter of the colonies was determined, and representative plates were photographed.

Results.—The relative rates of growth of the several isolates were so similar on the two media that the data are averaged. For two series on each medium the average diameter of the isolates in millimeters, in the order 1 to 8, were: 23, 23, 12, 13, 36, 20, 22, 27. The isolates differed greatly in growth rate, as indicated by this test.

Figure 5 illustrates the differences in gross colony characters of the isolates on malt agar. Attention is invited to the fact that the isolates had been carried in culture for a year and a half when these photographs were made. All except number 5, which was sporulating sparsely, were producing conidia abundantly when photographed. Non-sporulating sectors

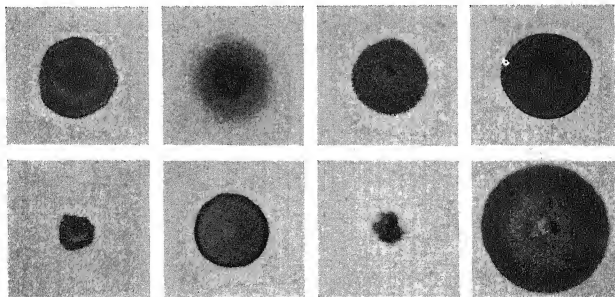


Fig. 5. Colonies of the eight monosporic isolates obtained from an ascus of *V. inaequalis*, after 24 days on malt extract agar at 16°C. In the top row from left to right are isolates 1, 8, 6, and 2, which comprise one sterility group; in the lower row, isolates 4, 7, 3, and 5, comprising the other group. The isolates are self- and intra-group sterile and inter-group fertile. The four isolates shown in the left half of the figure induced typical, sporulating lesions on McIntosh leaves, while those on the right did not (see text).

similar to those reported by Palmiter (1934) sometimes occurred, more frequently in certain isolates than others.

DISCUSSION.—The breeding experiments with the eight monascospore isolates from an ascus show that *V. inaequalis* is heterothallic in the sense that the isolates studied fall into two groups of four each with reference to sterility, being hermaphroditic, self-sterile, intra-group sterile, and inter-group fertile. The fact that the eight isolates fall into two groups of four each with reference to sterility and pathogenicity, respectively, clearly indicates that a process of segregation of genetic factors precedes ascospore formation and justifies the conclusion that both the antheridium and the ascogonium contribute hereditary materials to the ascospores. Combinations are, therefore, a major source of heritable variations in this pathogen. The question of heritable variations that may arise in the vegetative stage of the organism remains to be further investigated.

The results thus far obtained in further studies (Keitt, Palmiter, and Langford, 1938) with sets of eight monascospore isolates from asci of *V. inaequalis* are in agreement with those reported in the present paper. It is recognized, however, that study of a wider range of material may reveal other relationships than have thus far been encountered.

SUMMARY

The eight spores of an ascus of *Venturia inaequalis* were isolated and cultured in vitro.

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VITAMIN B₁ IN THE NUTRITION OF FOUR SPECIES OF WOOD-DESTROYING FUNGI¹

Norbert L. Noecker

THIS PAPER presents the results obtained from a study of the nutrition of the following fungi: *Stereum frustulosum* (Pers.) Fr., *Hydnum erinaceus* Bull., *Polyporus Spraguei* Berk. and Curt., and *Fomes ignarius* Fr., all of which are causally related to decay in the heartwood of living trees.

In a preliminary study it was found that none of these fungi grew well on a synthetic medium consisting of the usual mineral salts, nitrogen in the inorganic form, and dextrose. Schopfer (1934) and Burgeff (1934) found that *Phycomyces Blakesleeanus* can not grow on a rigorously synthetic medium unless vitamin B₁ is added. Intermediates of vitamin B₁ have been shown to be as effective as the vitamin itself in the nutrition of the same species of *Phycomyces* by Robbins and Kavanagh (1937).

While the present work was under way Kögl and Fries (1937) reported vitamin B₁ to be essential in the nutrition of the following Basidiomycetes: *Polyporus adustus*, *P. abietinus*, *Fomes pinicola*, *Trametes cinnabarina*, *T. serialis*, and *Lenzites sepiaria*. Vitamin B₁ was the only accessory growth substance required by *P. adustus* and *P. abietinus*; additional substances furnished by yeast extract were beneficial to the growth of the other four species of fungi, particularly the two last mentioned.

For a more comprehensive treatment of the literature concerning the rôle of vitamins in plant development the reader is referred to the review article by Bonner (1937).

MATERIALS AND METHODS.—Cultures of the fungi² used in this investigation were furnished as transfers from stock cultures maintained by the Division of Forest Pathology, Bureau of Plant Industry, Washington, D. C. Originally the cultures were obtained as isolates from the tissue of sporophores of these fungi.

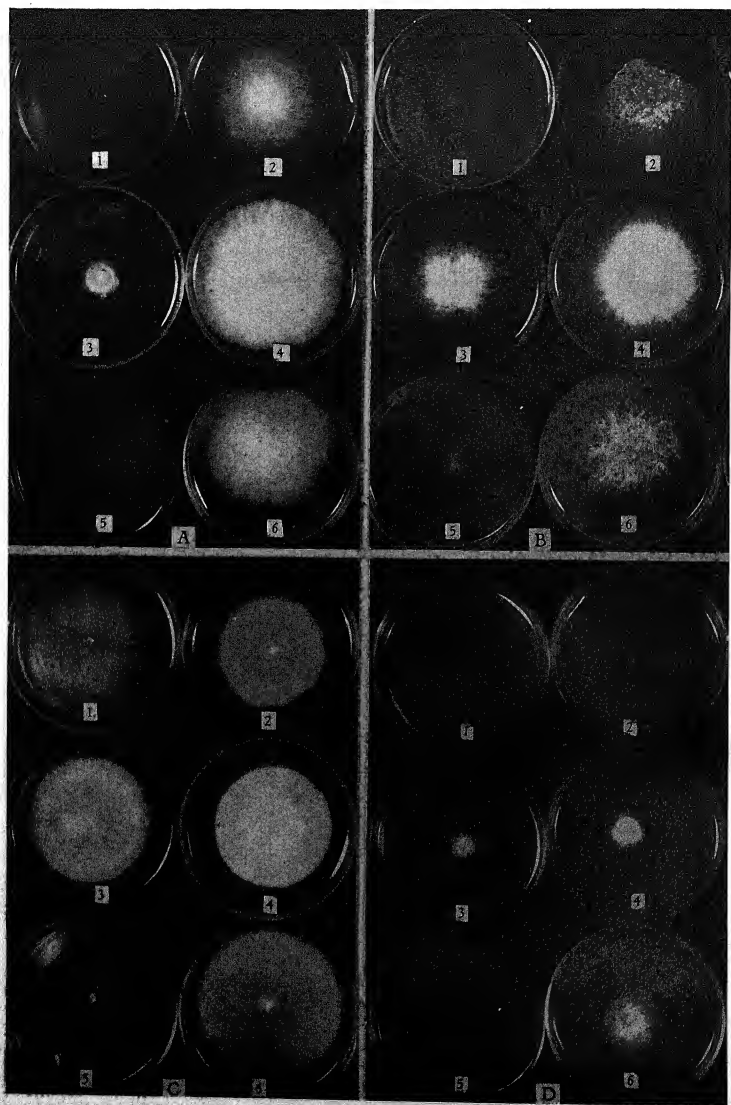
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The writer gratefully acknowledges the helpful advice and criticism given by Dr. B. M. Duggar and various members of the Bureau of Plant Industry during the progress of this work and in the preparation of this manuscript.

² The serial numbers assigned by the Bureau of Plant Industry are as follows: *S. frustulosum* (116), *H. erinaceus* (56482-S), *P. Spraguei* (14857-S), and *F. ignarius* (57049-S).

Cultures of these fungi were grown in Petri dishes on agar media which varied in respect to the addition of vitamin B₁ and the source of nitrogen employed. The sources of nitrogen used were as follows: peptone (Park, Davis & Co. Bacteriological), ammonium sulphate, and asparagine; each at a rate calculated to supply 2.8 grams of nitrogen per liter of medium. The three media, each containing a different source of nitrogen, were used with and without the addition of vitamin B₁ (Winthrop preparation of natural origin). When added, the vitamin was supplied at the rate of 3 mg. per liter of medium. The three nitrogen sources were respectively added to the following basic medium: dextrose, 90.0 g. (0.5 M); KH₂PO₄, 6.8 g. (0.05 M); MgSO₄·7H₂O, 2.46 g. (0.01 M); FePO₄ (1.0 per cent solution), 10 drops (trace); Bacto-agar (Difco), 20.0 g. (2.0 per cent); and distilled water, to make 1000 cc. of solution. The chemicals used were of reagent grade. The pH of these media was determined by the spot method, recommended by Felton (1921), and found to be 4.4. As one type of control, two standard media, commonly used for the culture of these fungi, malt extract (Trommer's) agar, pH 5.4, and potato dextrose agar, pH 5.2, were employed. The medium was added to 9 cm. Petri dishes in amounts of 10 cc. per dish and inoculated. The inoculum consisted of cylinders of agar, 1-3 mm. in volume, cut from the margins of young cultures. These were made by transfer from the stock cultures to potato dextrose agar in Petri dishes. The cylinders were cut from these 3 to 4 days after transfer. The cultures, in duplicate, were incubated at 25°C. for 12 days. At the end of this time the cultures were measured. The increase in diameter was used as the criterion of their growth rate. There was always a very close agreement between the duplicate cultures, and upon repetition of the experiment similar results were obtained. The results are presented in table 1.

EFFECT OF VITAMIN B₁ ON RATE OF GROWTH.—Vitamin B₁ augmented the growth of each species of fungus when added to the media. The amount of increase in the growth rate resulting from the addition of the vitamin varied with the species. This point is well illustrated by the photographs of the cultures shown in figure 1.



The greatest response to vitamin B₁ made by any species was that of *S. frustulosum* (fig. 1, A). Only when vitamin B₁ was supplied did this species make any growth on either the ammonium sulphate or asparagine media. When vitamin B₁ was added to the peptone medium an increase in growth rate of 400 per cent was observed. On this medium the growth of this species was comparable with that obtained on the malt extract or potato dextrose agars. In order to determine whether this species would maintain the same growth rate on these media, subcultures were made on similar media. The media for the subcultures differed from the originals only in the vitamin content. The vitamin B₁ was supplied as "Betabion Merck," a synthetic product; the concentration was reduced to 0.1 mg. per liter. The subcultures continued to grow at the same rate as the originals. The fact that the growth rate of this species on peptone plus vitamin B₁ continued at a rate comparable with that on malt extract agar indi-

trose agar is difficult to account for from the evidence at hand.

The response of *F. igniarius* (fig. 1, D) to the addition of vitamin B₁ was definite but not so great as that of the two species mentioned. On the ammonium sulphate medium growth occurred only when vitamin B₁ was added. On the asparagine medium there was no growth, either with or without the addition of the vitamin. On peptone there was a very slight increase in the growth rate when the vitamin was supplied; however, the growth rate on this medium did not approach that on the potato dextrose or malt extract agars. Evidently the malt and potatoes furnish one or more additional factors required for the growth of this species.

P. Spraguei (fig. 1, C) was, among the fungi studied, the least affected by vitamin B₁, as far as radial growth was concerned; nevertheless, there were differences in the densities of the mycelial mats. Although the cultures on the ammonium sulphate and aspara-

TABLE 1. Growth of wood-destroying fungi on agar media, nitrogen being supplied in various forms with and without the addition of vitamin B₁.

Media	Diameter of colony, 12 days old, in cms.							
	<i>H. erinaceus</i>		<i>F. igniarius</i>		<i>P. Spraguei</i>		<i>S. frustulosum</i>	
	Control	B ₁ added	Control	B ₁ added	Control	B ₁ added	Control	B ₁ added
Asparagine	4.6 ^a	5.6	0.0	0.0	5.2	5.9	0.0	4.8
Peptone	4.7	7.0	1.8	2.2	6.4	7.1	1.9	7.8
(NH ₄) ₂ SO ₄	1.6	6.0	0.0	3.1	4.2	6.8	0.0	7.1
Malt extract ...	9.1		6.4		13.1 ^b		8.3	
Potato dextrose ..	5.4		4.2		9.6		6.4	

^a The numbers denote averages of four cultures.

^b Numbers exceeding 9 cm. were obtained from calculations based upon the average daily increment of growth made by the fungus in covering the 9 cm. Petri dish.

icates that no accessory growth substances other than vitamin B₁ is required when peptone is used as the source of nitrogen.

The three other species of fungi exhibit less marked, yet distinct, responses to the addition of vitamin B₁. Of the three other species, *H. erinaceus* (fig. 1, B) exhibits the greatest effect of vitamin B₁ on the rate of growth. This species was comparable with *S. frustulosum* in that only when vitamin B₁ was supplied did growth occur on either the ammonium sulphate or asparagine media. When vitamin B₁ was added to the peptone medium an increase in growth rate of 50 per cent resulted. The rate of growth on this medium surpasses that on potato dextrose agar but is inferior to that on malt extract agar. The relatively poor growth of this species on potato dex-

trase media grew without the addition of vitamin B₁, some improvement was noted when the vitamin was added. On peptone, regardless of differences in vitamin B₁ content, the cultures were approximately the same diameter; they varied only in the density of the mycelium. The growth on this medium was much poorer than that on the potato dextrose or malt extract agars.

CARBON NUTRITION.—Dextrose was utilized readily as the source of carbon by the four species of fungi investigated; however, xylose appeared to be superior for *S. frustulosum*, the only species studied further concerning carbon nutrition. When xylose was substituted for dextrose in the peptone, ammonium sulphate, and asparagine media containing vitamin B₁,

Fig. 1. Cultures of fungi grown for 12 days on agar media which differ both in respect to the source of nitrogen and the vitamin B₁ content: A, *S. frustulosum*; B, *H. erinaceus*; C, *P. Spraguei*; D, *F. igniarius*. The right hand culture (2, 4, 6) of each species was provided with vitamin B₁; the left hand culture (1, 3, 5) is the corresponding control. The nitrogen sources were: top row (1, 2) asparagine; middle row (3, 4) peptone; bottom row (5, 6) ammonium sulphate.

the average increase in growth for 12 cultures was 49 per cent.

DISCUSSION.—The results indicate that vitamin B₁ is an accessory growth substance required in the nutrition of the four species of fungi investigated. For one species, *S. frustulosum*, vitamin B₁ appears to be the only accessory growth factor required, except for some which may possibly be supplied by peptone. For the other species, particularly *F. ignarius*, other factors furnished by malt extract are beneficial. The extracts of malt, yeast, and other plant products contain, in addition to vitamin B₁, a number of other substances, some of which have been identified as nutrients essential in plant nutrition. Yeast extract contains, in addition to vitamin B₁, biotin and inositol, all of which are required in the nutrition of *Trametes serialis* according to Kögler and Fries (1937). Extracts of malt and yeast contain a number of amino acids, which White (1937) has reported to be beneficial in the nutrition of his cultures of excised tomato roots.

The accessory growth substances contained in peptone are not so well known as those in yeast. The brand of peptone employed in this investigation was very low in vitamin B₁ content, as shown by the fact that *S. frustulosum* did not grow well on this medium

without the addition of the vitamin. The relative importance of the amino acids and the more complex nitrogen compounds present in peptone remains to be determined.

In regard to the nitrogen nutrition it should be noted that all the fungi used in this investigation were able to utilize inorganic nitrogen when vitamin B₁ was supplied, making it possible to culture them on a strictly synthetic medium. The inability to culture the wood-destroying fungi on such a medium in the past has hindered the study of carbon and nitrogen nutrition.

SUMMARY

Vitamin B₁ is necessary for the growth of *S. frustulosum*, *H. erinaceus*, *P. Spraguei*, and *F. ignarius* on a medium composed of chemically pure ingredients. There is considerable variation, however, among these fungi in their responses. Nitrogen may be supplied in either the inorganic or the organic form, the more complex being the more effective. Dextrose may be utilized as a source of carbon, but xylose is superior for *S. frustulosum*, the only species of fungus studied in this connection.

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CULTIVATION OF EXCISED ROOTS OF DICOTYLEDONOUS PLANTS¹

Philip R. White

THE CHARACTERISTIC behavior of excised tomato roots in culture has been clearly established by a long series of experiments under a variety of conditions which need not be reviewed here. This is the only species of plant, however, whose roots have been grown for any considerable period. Robbins and Bartley (1937a, 1937b), and Fiedler (1936) have also successfully grown tomato roots, but the fact that none of these authors succeeded in obtaining continuous growth of maize roots (Robbins and V. B. White, 1936; Robbins, V. B. White, McClary, and Bartley, 1936; Fiedler, 1936) indicates that, under given conditions, roots of other species of plants can be expected to behave differently. The suggestion has been made that the tomato root may be an exception in its capacity to grow as an isolated organ. A comparison of the behavior of excised roots of

species other than tomato has, therefore, seemed desirable. This paper presents results obtained in efforts to grow the roots of a number of species in pure culture.

The methods used in securing excised roots from seedlings and in culturing them after excision were the same as those outlined elsewhere (White, 1934). The nutrient solution was that previously described (White, 1934). It contained the salts of a modified Uspenski solution, 2 per cent. sucrose, and 0.01 per cent. extract of dried brewers' yeast. Roots were considered satisfactorily established when they had been grown for at least 10 passages of a duration of one week each without significant diminution in growth rate. There seems to be little doubt that roots which have fulfilled this requirement can be grown indefinitely.

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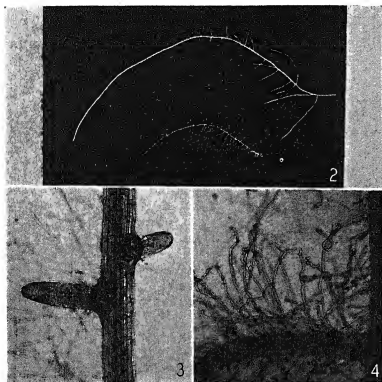


Fig. 2-4.—Fig. 2. Roots of *Lycopersicon esculentum* above, of *Fagopyrum esculentum* below. $\times 0.75$ —Fig. 3. Branching habit of *Fagopyrum esculentum*. $\times 30$ —Fig. 4. Root hairs of *F. esculentum*. Note the contorted habit. $\times 30$. (Photographs by J. A. Carlile.)

Roots of 30 species in addition to tomato, representing eight families of dicotyledonous plants, were tested. The classification used throughout has been that of the Index Kewensis (1895).

RESULTS.—The results of all experiments are summarized in table 1. Out of the 31 species tested only twelve proved refractory to the methods used. Fifteen species were cultivated for 10 or more passages. Four additional species, although giving promise of ability to grow indefinitely, were discarded after from 5 to 8 passages because lack of space made it necessary to limit the number of cultures maintained. Of the fifteen species successfully established 3 were discarded after being maintained for from 11 to 13 passages. Three more were accidentally lost after having been maintained for 20, 23, and 53 passages. Nine species are still growing with undiminished vigor after 40 weeks or more in culture. The increment rates of these nine species, over the period September 15, 1937, to January 15, 1938, are presented in figure 1. Although growth rates in all cases showed a slight seasonal decrease over this period (see White, 1937) they have been, in general, remarkably constant.

Roots of these nine species can be separated roughly into two groups, those that grew slowly (1–3 mm. per culture per day): *Fagopyrum esculentum* Mönch, *Nicotiana langsdorffii* Weinm., *Daucus carota* L., *Callistephus hortensis* Cass. (= *C. chinensis* Nees),

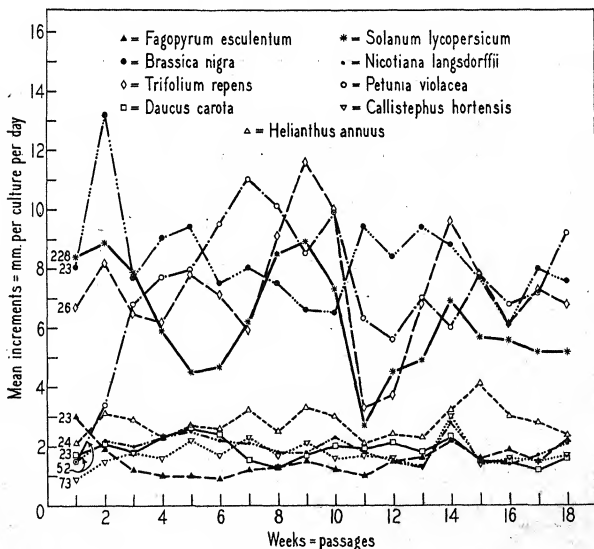


Fig. 1. Growth rates of nine species of plants over the 18 week period, September 15, 1937, to January 15, 1938. The number opposite the beginning of each curve represents the number of passages through which roots of the species represented had been carried up to the passage designated in the graph as "1." Thus tomato roots had been maintained, up to January 15, for $228 + 18 = 246$ passages, mustard roots for $23 + 18 = 41$ passages, etc.

TABLE 1. *Growth of excised roots of dicotyledonous plants.*

No.	Common name	Genus	Species	Family no.	Family	Mean growth rate in mm. per culture per day	Maximum no. of passages Mar. 1, 1938	Condition			Prognosis	
								Excellent	Fair	Poor	Excellent	Doubtful
1	Buckwheat	Fagopyrum	esculentum	1	Polygonaceae	1.3	41		+		+	
2	Beet	Beta	vulgaris	2	Chenopodiaceae	—	4	**				+
3	Radish	Raphanus	sativus	3	Cruciferae	13.5	20**					**
4	Mustard	Brassica	nigra		"	6.2	41	+			+	+
5	Cabbage	"	oleracea		"	—	3					+
6	Kohl-rabi	"	"		"	—	4					+
7	Broccoli	"	"		"	—	4					+
8	Alfalfa	Medicago	sativa	4	Leguminosae	6.3	13*	*			*	*
9	Sweet clover	Melilotus	alba		"	2.6	8*		*		*	*
10	White clover	Trifolium	repens		"	8.2	44	+			+	
11	Red clover	"	pratense		"	5.9	23**	**				**
12	Vetch	Vicia	sp.		"	6.2	6*	*			*	
13	Lupine	Lupinus	sp.		"	—	2					+
14	Soy bean	Glycine	soja		"	1.4	11*			*		*
15	Carrot	Daucus	carota	5	Umbelliferae	1.8	41		+	+	+	+
16	Celery	Apium	graveolens		"	0.7	5*		*		+	
17	Tomato	Lycopersicum	esculentum	6	Solanaceae	6.2	231	+			+	
18	Potato	Solanum	tuberosum		"	—	2					+
19	Egg plant	"	melongena		"	—	4					+
20	Jimson weed	Datura	stramonium		"	1.8	11*			*		*
21	Tobacco	Nicotiana	tabacum		"	2.6	53**		**			**
22	—	"	lansdortii		"	1.7	74		+		+	+
23	—	"	sylvestris		"	—	7					+
24	—	"	glutinosa		"	—	7					+
25	—	"	rustica		"	—	1					+
26	Petunia	Petunia	violacea		"	5.4	74		+		+	+
27	Cucumber	Cucumis	sativus	7	Cucurbitaceae	—	1					+
28	Sunflower	Helianthus	annuus	8	Compositae	2.9	42	+			+	*
29	Chicory	Cichorium	sp.		"	0.9	6*		*			+
30	Lettuce	Lactuca	scariola		"	—	5					
31	Aster	Callistephus	chinensis		"	1.2	95		+			+

* Discontinued July 8 to conserve space. Alive and growing at that time.

** Died after sudden change of temperature in first week of September. Condition up to that time as indicated.

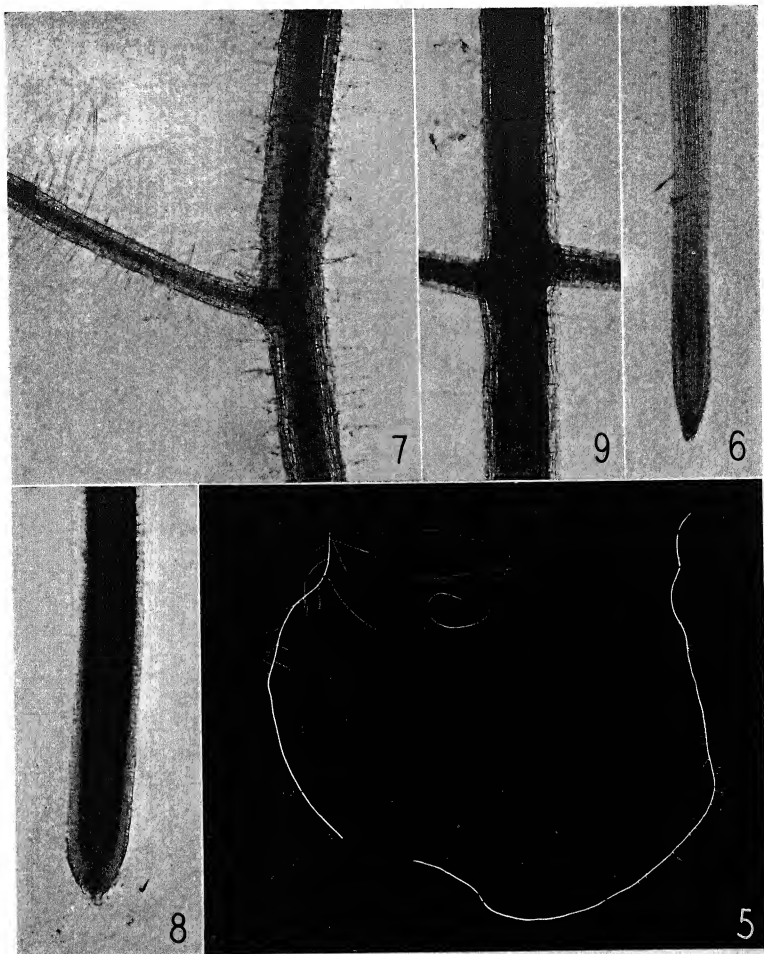


Fig. 5-9.—Fig. 5. At the left, control (*Lycopersicum esculentum*); center, *Brassica nigra*; right, *Raphanus sativus*. The mustard root was too translucent to show up well in the photograph. Note the difference in branching habit between roots of the two crucifers. $\times 0.66$.—Fig. 6. Root tip of *Brassica nigra*. The root cap is almost wanting. $\times 36.5$.—Fig. 7. Branching habit of *Brassica nigra*. Note the root primordium (black spot) on the upper side of the branch at the left. $\times 36.5$.—Fig. 8. Root tip of *Raphanus sativus*. Like that of *B. nigra*, the root-cap is almost completely wanting, but the normal diameter of the root is much greater in *R. sativus* than in *B. nigra*. $\times 36.5$.—Fig. 9. Branching habit of *R. sativus*. The branches are very slender in comparison with the main root. $\times 36.5$. (Photographs by J. A. Carlile.)

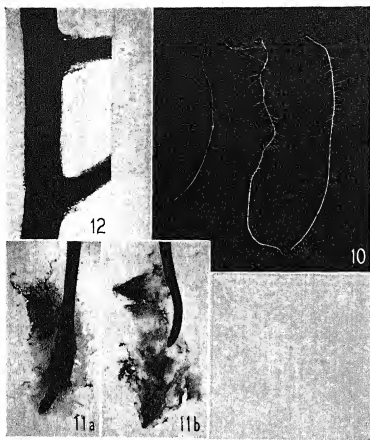


Fig. 10-12.—Fig. 10. At the left, control; center, *Trifolium repens*; right, *T. pratense*. Roots of both species of clover grew more rapidly than did those of tomato. $\times 0.33$.—Fig. 11. Root tips of *Trifolium pratense*. a. The tip of the large root-cap has been pushed off and is to be seen at the left. A loose mass of cells has replaced it but is not yet consolidated into a typical root-cap. The basal portion of the old cap remains as a sheath around the root. Such remnants are often evident many centimeters back of the growing point. b. At the bottom and left, 3 large, conical root-caps. On the root is a fourth cap not yet fully developed. These root-caps are easily visible with the naked eye and are a very striking feature of roots of legumes. $\times 6$.—Fig. 12. Branching habit of *Trifolium repens*. Note the characteristic collar around the base of each branch root. $\times 18$. (Photographs by J. A. Carlile.)

and *Helianthus annuus* L., and those that grew rapidly (4-10 mm. per culture per day): *Brassica nigra* Koch, *Trifolium repens* L., *Petunia violacea* Lindl., and *Lycopersicon esculentum* Mill. The detailed behavior differed so widely from species to species that each will be described separately.

Polygonaceae.—*Polygonum esculentum* Mönch. — Buckwheat roots were grown at a fairly uniform but low rate (fig. 1) for more than 40 passages. The growth habit was much like that of tomato (fig. 2), but the roots were much more slender and flexible.

They were profusely branched and covered with long, loosely matted, irregularly contorted, and sometimes even forked root hairs which gave them a striking furry appearance (fig. 2, 3, 4). The behavior of the root hairs (fig. 3, compare with fig. 15 and 16, *Nicotiana langsdorffii*) strongly suggested that the nutrient was in some way slightly injurious. Root-caps and all old tissues turned a bluish gray color. These roots appeared to be capable of growing for indefinite periods in the solution developed for tomato roots, even though they were not in the best possible condition.

Chenopodiaceae.—*Beta vulgaris* L. — Beet roots when first isolated grew well. Upon subculturing, however, the newly formed portions became excessively slender. They soon ceased to branch, formed few root hairs, and finally died. No cultures were kept alive for longer than four passages. The roots took on the deep purple color characteristic of red beets, the color shading from white to purple with increasing age of the tissue. This color developed equally early, regardless of whether the roots were grown in daylight or in darkness. The culture conditions suitable for tomato are evidently unsatisfactory for beet roots. It is possible that some sugar other than sucrose may be necessary for the culture of this species.

Cruciferae. Five varieties of crucifers, representing 3 species, were tested. Roots of 3 varieties of *Brassica oleracea* L. (cabbage, broccoli, and kohlrabi) all failed to grow for more than 3 or 4 passages. Roots of *Brassica nigra* Koch (mustard) and of *Raphanus sativus* L. (radish), on the other hand, grew excellently. Those of *R. sativus* were accidentally lost in the 23rd passage. Those of *Brassica nigra* were maintained for more than 40 passages without significant diminution in growth rate. They were very slender and translucent, hence difficult to photograph (fig. 5, center). They branched profusely. Apical dominance appeared to be very weak in this species so that the branches soon overtook the main root in growth. In contrast with certain other species (see *Trifolium repens*) these branches were as easily established as subcultures as was the primary tip. Large numbers of cultures could thus be obtained from a single root without difficulty. Roots of *Brassica nigra* should prove to be especially favorable material for many types of experimental work (fig. 5, 6, 7). Roots of *Raphanus sativus* (radish) likewise grew rapidly, but they did not branch as profusely as did those of mustard (fig. 5, 8, 9). Bulbous roots were not formed.

Fig. 13-16.—Fig. 13. Roots of solanaceous plants. Below, control (*Lycopersicon esculentum*). Upper row, reading from left to right: *Nicotiana tabacum*, *N. langsdorffii*, *Petunia violacea*, and *Datura stramonium*. The characteristic long root hairs on *N. langsdorffii* blur the outline of the photograph. The peculiar cross banding on the root of *Datura stramonium* is produced by short compact root hairs whose length depends on the time of day when formed so that each band represents one 24 hour period. $\times 1$.—Fig. 14. Root tip of *Lycopersicon esculentum*. $\times 25$.—Fig. 15. Root tip of *Nicotiana langsdorffii*. Note how close behind the growing point the first root hairs develop. The so-called "region of elongation" is very short. $\times 36.5$.—Fig. 16. Branching habit of *Nicotiana langsdorffii*. $\times 36.5$. (Photographs by J. A. Carlile.)

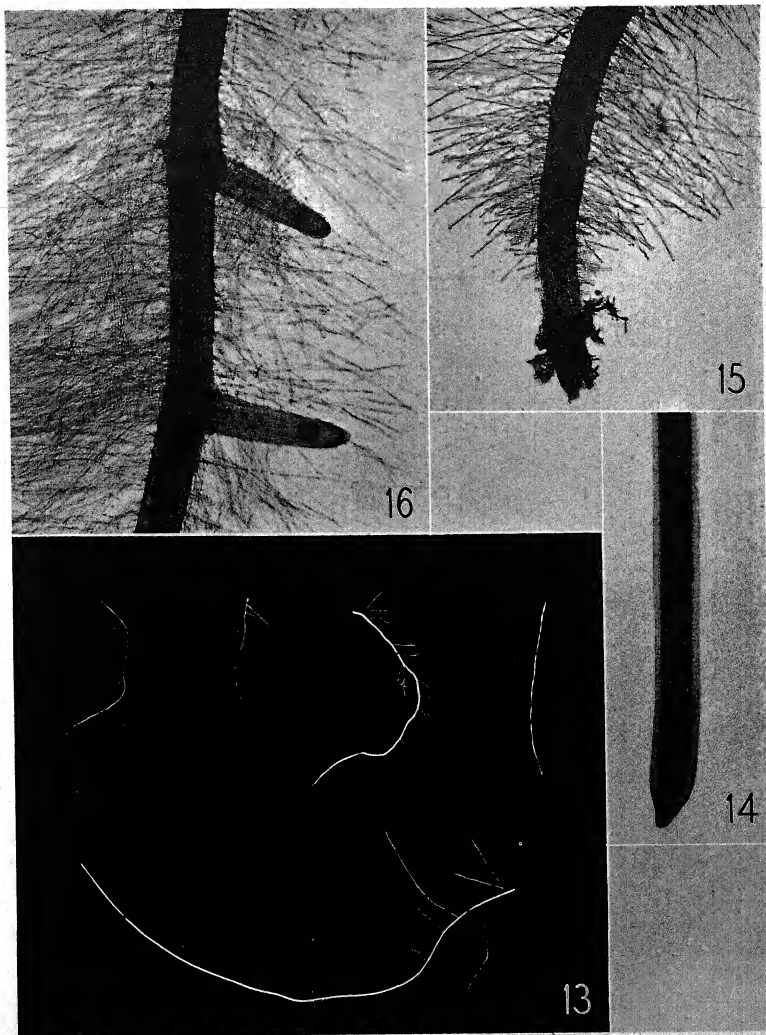
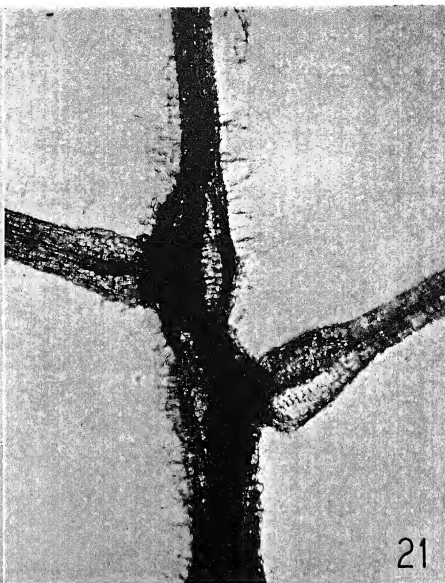


Fig. 13-16. Roots of solanaceous plants. See foot of page 352.



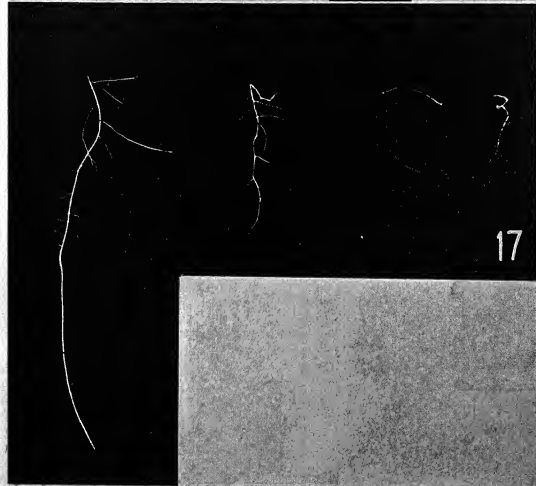
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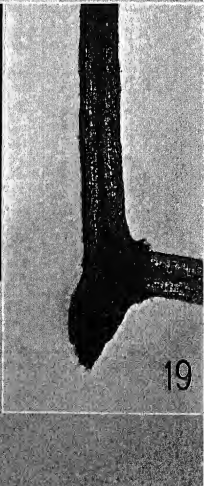
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Leguminosae.—Seven species of legumes—alfalfa (*Medicago sativa* L.), white sweet clover (*Melilotus alba* Desr.), white clover (*Trifolium repens* L.), red clover (*T. pratense* L.), soybean (*Glycine soja* Sieb. et Zucc.), vetch (*Vicia* sp.), and lupine (*Lupinus* sp.)—were tested. All but one, the lupine, grew satisfactorily. The growth rates were high (6–8 mm. per culture per day) in all species except sweet clover and soybean (table 1). The growth habit of all species was very characteristic. The roots were long, clean, fairly large but much bent (fig. 10). They branched profusely, but the branches for the most part remained short. Great difficulty was encountered in establishing branches as subcultures (compare Bonner and Addicott, 1937). Apparently apical dominance, which is present but easily suppressed in most species and is almost wanting in roots of *Brassica nigra*, is a pronounced and rigidly established characteristic of these legumes. When once upset (by severing the tip) some time was required to establish a new dominance in a branch. Nevertheless, satisfactory clones of the six species mentioned above were established. Four of them were discarded after six or more passages, the strain of *Trifolium pratense* was accidentally lost after 26 passages, and only *Trifolium repens* was retained. This has been carried through more than 40 passages without significant diminution in growth rate (fig. 1). Perhaps the most striking feature of the legume cultures was the enormously developed and almost invariably dark colored root-caps. These sometimes formed distinct sheaths enclosing the meristem for as much as 6 or 8 mm. Figure 11a shows the enormous development of the cap on a root of *Trifolium pratense*. Figure 11b illustrates a root of the same species which had sloughed off 3 root-caps and was developing a fourth. Another peculiarity of legume roots was the habit of forming characteristic collars around the bases of the branches (fig. 12). This does not occur on roots of any other family of plants studied. There appeared to be some definite relation between the presence of the root-cap and the vigor of the culture, for, when such caps were not developed, growth of the root was always poor. Many roots, as they grew older, became discolored with either brown or blue-gray pigments. Legume roots seem to be fairly satisfactory as culture material and should prove useful for *in vitro* studies of the nitrifying bacteria.

Umbelliferae.—Roots of *Daucus carota* L. (carrot) and *Apium graveolens* L. (celery) were cultured for considerable periods but branched sparingly and grew very slowly (table 1). The celery roots were dis-

carded after 5 passages, to conserve space, but those of carrot were maintained for more than 40 passages (fig. 1).

Solanaceae.—Because of the extensive use of solanaceous plants in the study of plant virus diseases, an especial effort was made to obtain a good representation of root cultures from species of this family. Nine species in addition to tomato were tested (table 1). *Nicotiana sylvestris* Spegaz. et Comes, *N. glutinosa* L., and *N. rustica* L. all proved refractory to the methods used and could not be maintained for more than a few passages. Roots of *N. tabacum* L. and *N. langsdorffii* Weinm. were satisfactorily established, but the former strain was accidentally lost in the 53rd passage. They were more slender than those of tomato but otherwise similar (fig. 13). Roots of *N. langsdorffii* were maintained for more than 70 passages (fig. 1). They also were slender and produced characteristic dense mats of long, straight root hairs (fig. 13, 15, 16; compare fig. 3 and 4). Roots of *Petunia violacea* Lindl. were likewise maintained for more than 70 passages with undiminished vigor. They were somewhat more slender than those of tomato and branched more profusely than either tomato or tobacco roots (fig. 13). The roots of *Datura stramonium* L. grew continuously but very slowly (table 1) and failed to branch at all (fig. 13). They were discarded after 11 passages. Roots of *Solanum melongena* L. and *S. tuberosum* L. proved completely refractory to the treatments tested. Those of the latter species were obtained from seedlings, from tubers, and from stem cuttings. A great variety of nutrients, at different pH's, containing various sugars, with and without addition of vitamin B₁, were tested both in diffuse light and in the dark, yet no cultures were kept growing for more than 2 passages. The contrast between this behavior and that of other members of the same family was very striking.

Cucurbitaceae.—*Cucumis sativus* L. (cucumber) was tested, but, like the potato, proved refractory to all treatments used. The roots grew well so long as they were attached to the seedlings but invariably failed within a few days after excision and could not be maintained for more than a single passage.

Compositae.—Four species of composites—*Callistephus hortensis* Cass., *Cichorium* sp., *Lactuca scariola* L., and *Helianthus annuus* L.—were tested. Roots of all except the lettuce grew, although slowly (table 1, fig. 1). Two distinct types of growth behavior are recognizable among them. Roots of aster and chicory were very slender and wiry, formed few root hairs, and seldom branched unless severed or otherwise in-

Fig. 17–21.—Fig. 17. Reading from left to right: control (*Lycopersicum esculentum*), *Helianthus annuus*, *Callistephus hortensis*, and *Cichorium* sp. Note the "zig-zag" habit of the sunflower root and the hyperhydric swollen areas on those of sunflower and chicory. The example of aster was a segment taken out of the middle of a root and carries a single branch root. $\times 0.75$.—Fig. 18. Root tip of *Callistephus hortensis*. $\times 36.5$.—Fig. 19. Branching habit of *Callistephus hortensis*. The single branch originates just back of the distal cut end and is of equal diameter with the parent root. $\times 36.5$.—Fig. 20. Root-tip of *Helianthus annuus*. The root is very slender. Note the long, rather dense mat of sloughed off root-cap cells. This is to be contrasted with the conical root-caps shown in figure 11. $\times 36.5$.—Fig. 21. Branching habit of *Helianthus annuus*. The cortical tissue has swelled and split away from the stele at the base of the branches and in the main root in the neighborhood of the branches. $\times 36.5$. (Photographs by J. A. Carlile.)

jured. Under these circumstances a callus formed at the point of injury or at the distal end of the fragment, and a single root developed at the base of the callus (fig. 19). The calluses on these roots often became very large. Sometimes callus-like areas resulting from hyperhydric swelling and rupturing of the cortex developed at irregular intervals along the roots. This behavior shows that the cultural conditions were not fully satisfactory for these species. Nevertheless, roots of aster have been maintained for over 100 passages (fig. 1). Roots of the sunflower, *Helianthus annuus* L., on the other hand, grew quite satisfactorily after an early refractory period (fig. 1, 17, table 1). They were slender but not wiry, with sparse root hairs. They branched fairly profusely, mostly in a single plane, and the main root was bent rather sharply at each branch so as to produce a very characteristic "zig-zag" habit (fig. 17, 21). This bending appears to be an expression of the habit, already mentioned as occurring in other composites, of forming calluses. The cortical tissues, especially at the bases of the branches, frequently became hyperhydric, rupturing and pulling away from the stele to form glistening white, swollen areas which forced the adjacent root tissues out of line (fig. 21). This habit was especially marked in the early passages and at times threatened to destroy the cultures entirely, but gradually decreased in frequency of occurrence. The sunflower root has been maintained through more than 50 passages and now seems to be well adjusted to its environment.

DISCUSSION.—Of the 31 species of plants tested, roots of seven species—*Raphanus sativus*, *Brassica nigra*, *Medicago sativa*, *Trifolium repens*, *T. pratense*, *Vicia* sp., and *Petunia violacea*—grew as well as, or better than, did those of *Lycopersicum esculentum* under the cultural conditions developed as suitable for the latter species. Roots of eleven other species—*Fagopyrum esculentum*, *Melilotus alba*, *Glycine soja*, *Daucus carota*, *Apium graveolens*, *Datura stramonium*, *Nicotiana tabacum*, *N. langsdorffii*, *Helianthus annuus*, *Cichorium* sp., and *Callistephus chinensis*—were likewise established but grew at slower rates. Twelve species were maintained for 20 or more passages without significant diminution in growth rate, and 9 species (fig. 1) have been maintained for more

than 40 passages. The capacity to grow as isolated units, therefore, is not restricted to roots of tomato, but is fairly widely distributed, at least in the dicotyledons. Hence, all the more interest attaches to the twelve species which were not successfully grown, and an explanation for this failure must be sought. It seems extremely unlikely that there can exist any fundamental difference between *Brassica nigra* and *B. oleracea*, for example, or between *Solanum lycopersicum* (= *Lycopersicum esculentum*) and *S. tuberosum* so great as to make it actually impossible to grow the roots of the one species when those of the other can be grown easily. Failure to accomplish this result with roots of *B. oleracea* and *S. tuberosum* is probably attributable to imperfections of technique rather than to any peculiarity of these species. Isolated roots are evidently much more rigid in their requirements than are whole plants. The leaves, stems, and older roots obviously act as buffers against variations in the environment, and in the absence of these parts the conditions must be very delicately balanced. The sensitiveness of isolated tomato roots to variations in temperature and pH have been demonstrated elsewhere. Evidently there are factors to which the roots of other species are equally sensitive, which have not yet been identified. While all species of plants should eventually lend themselves to this technique, if only the right conditions can be found, it is not possible at present to make any generalizations as to the nature of these conditions.

SUMMARY

Attempts were made to grow isolated roots of 30 species of dicotyledonous plants in vitro. Eighteen were grown successfully, while 12 proved refractory to the treatments used. Although roots of all species of dicotyledons can probably be grown under proper conditions, it appears that these conditions will, in many cases, differ widely. Apparently no generalizations can be drawn as regards the requirements of isolated roots which will apply satisfactorily to all species.

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THE IDENTIFICATION AND ESTIMATION OF ETHYLENE IN THE VOLATILE PRODUCTS OF RIPENING BANANAS¹

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THE HISTORY of this subject may conveniently be divided into three phases: first, the early work which led to the discovery that ethylene had some influence on the rate of ripening of fruits and vegetables; second, the finding that plant tissue apparently gives off some gas or vapor which affects the ripening of fruits; and last, the identification of this active substance.

Forced curing of citrus fruits was practiced in California, and the early use of a tent and a few kerosene stoves in the field was considered ample to provide the "heat" necessary for stimulation of the coloring of the fruit. After sad experience had shown that steam heat was not so effective as the "crude" earlier arrangement, Sievers and True (1912) demonstrated that the coloration in forced curing was brought about primarily by the gaseous combustion products of the kerosene stoves. Chace and Denny (1924) and Denny (1924) proved by a series of experiments that the effective constituent among these gases was readily absorbed by bromine and had a specific gravity approximately equal to that of air. These facts suggested that the active gas might be ethylene, and further work proved ethylene to be very effective in bringing about the desired change in the color of the fruit.

Since then Denny (1924), Harvey (1925), Chace and Church (1927), Regeimbal and Harvey (1927), Mack (1927), Regeimbal, Vacha, and Harvey (1927), Hibbard (1932), Dufrenoy (1929), Kohman (1931), Wolfe (1931), Rosa (1928), Ramsay and Musso (1930), Fudge (1930), Davis and Church (1931), Marloth (1933), and Dustman (1934) have reported the effects of ethylene upon the ripening of various plant products.

The experience of the United Fruit Company in the carrying of bananas from the tropics to this country may have parallels in commercial handling of fruits, though none has been published. Over a period of years prior to 1917, it was noticed that some bananas ripened faster than the bulk of the shipment during transportation. It was further noted that this ripening generally occurred in pockets. Moreover, there was a tendency for a "ripe pocket" to act as a focus of ripening for a whole bin of bananas.

Evidence accumulated which pointed to the fact that this pocket ripening was not entirely caused by heat, and it seemed that some other factor helped to induce this phenomenon. The theory developed that the carbon dioxide evolved by the fruit during respiration was influential in the production of "ripes" in pockets. Though this was soon discredited, there seemed to be some correlation between the respiration rate and accelerated ripening.

To overcome this, a ventilating system for refrigerator ships was designed specifically to provide controlled air changes in a manner which would eliminate gas pockets. In 1917 it became the practice to measure the carbon dioxide content of the air to secure an index to regulate the ventilation. Determination of carbon dioxide is still used for that purpose, even though it has been proved that carbon dioxide has no accelerating action on the ripening. In 1921, Mr. V. W. Ridley (1923) of the United Fruit Company demonstrated the presence of a then unknown gas in the respiration products of ripe bananas which caused the acceleration of the ripening of green bananas. No conclusions were reached at that time concerning the possible identity of this gas.

Early in 1932 Elmer reported that a gas emanating from apples inhibited the sprouting of potatoes, leading to further work by Heulin (1933), Smith and Gane (1933), and Kidd and West (1934), whose results indicated a marked similarity between the effects produced by the volatiles of ripe apples and those shown by low concentrations of ethylene. It was these publications that pointed to the hypothesis that ethylene is produced by plant tissue during ripening.

A very useful method for investigators in this field has been developed out of the observation that the petioles of many plants, when exposed to an atmosphere bearing ethylene, grow more rapidly on the upper side than on the lower side. This is also true when the atmosphere contains other olefines, artificial illuminating gas, or tobacco smoke. This phenomenon called epinasty is a selective stimulation of growth of the upper portion of the leaf petiole, causing the petiole to incline downward. In view of the fact that a very excellent summary of early work and of their own contributions was made by Crocker, Zimmerman, and Hitchcock (1932), no attempt will be made to give an exhaustive treatment of the results of earlier workers.

For the purpose of the present study, the most important conclusion reached by Crocker and his co-workers is that only five gases of thirty-eight tested caused epinasty of tomato plants. These were ethylene, acetylene, propylene, carbon monoxide, and butylene. Further, if the minimum concentration of ethylene required to induce epinasty was considered as one, the required concentrations of the others were acetylene and propylene, 500; carbon monoxide, 5,000; and butylene, 500,000.

Denny and Miller (1935) and Denny (1935) tested many plant tissues for emanations causing epinasty of potato plants. On the basis of the work of Crocker et al., cited above, and that of Gane (1934), who by absorption of the volatile of apples in bromine secured

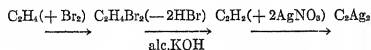
¹ Received for publication March 19, 1938.

and identified a small amount of ethylene dibromide, these workers concluded that the gas evolved by plant tissue was probably ethylene, and that any evidence that some other chemical was responsible for the phenomena observed would have to be accompanied by proof that ethylene was not present in a concentration of one part in 20 million parts of air.

Later, Elmer (1936) also presented evidence indicating the probability that ethylene was given off by ripe apples.

The object of the present work was the chemical identification and estimation of the active gas, probably ethylene, given off during ripening of bananas. Since the concentration of the active gas in the atmosphere about normally ripening fruit was estimated to be in the order of one part in 10^6 or 10^7 of air, the application of micro-chemical methods appeared advisable.

The method employed consists of converting the ethylene to ethylene dibromide, which in turn is converted to acetylene, the latter being conclusively identified as silver acetylide, as shown in the following equation:



PROCEDURE.—The first step was to devise a suitable absorption method. The small amount of ethylene in large volumes of air precluded the possibility of using any of the standard methods which would involve its isolation or concentration as a gas. It was therefore necessary to select a procedure which could be adapted to a micro scale.

Trial runs employing bromine dissolved in petroleum ether (Plotnikow, 1905), bromine solutions in carbon tetrachloride (Davis, 1928; Mitsujuri, 1933), bromine in potassium bromide solutions, and pure bromine (Oberseider and Boyd, 1931; Scott, 1927; Mitsujuri, 1933) as absorption media were made. The results indicated that pure liquid bromine is most efficient.

Inasmuch as the vapor pressure of bromine at room temperature is high, the use of ordinary types of absorption bottles leads to excessive loss of bromine. Furthermore, since the amount of ethylene dibromide to be expected is extremely small, it was thought advisable to reduce the amount of bromine required as the absorption medium. Since no standard gas-washing vessel entirely meets these requirements, a unit was designed for the purpose (Brenner and Poland, 1937). This apparatus (fig. 1) consists of a side-arm test tube into which is fitted the delivery tube of a spiral condenser. This condenser has a fritted-glass diffusing plate at its lower, delivery end and a removable head with an outlet tube.

The volume of bromine required is only 20 to 50 ml., and the jacket around the spiral makes it possible to minimize the loss of bromine by circulating brine at -5° to $0^\circ\text{C}.$ around the spiral. The long path of travel and the intimate contact effected

assure nearly quantitative absorption. The efficiency of absorption of known dilute mixtures of ethylene and air was found to be approximately 95 per cent.

The absorption train (fig. 3) contained the following units in series: (1) a gas-washing bottle containing liquid bromine; (2) a freezing trap immersed in a solid carbon dioxide-alcohol bath; (3) a gas-washing bottle containing a 30 per cent potassium hydroxide solution; (4) a gas-washing bottle containing a 5 per cent potassium iodide solution; (5) four 55-gallon drums in parallel, each holding 100 pounds of bananas; (6) a large drying bottle filled with calcium

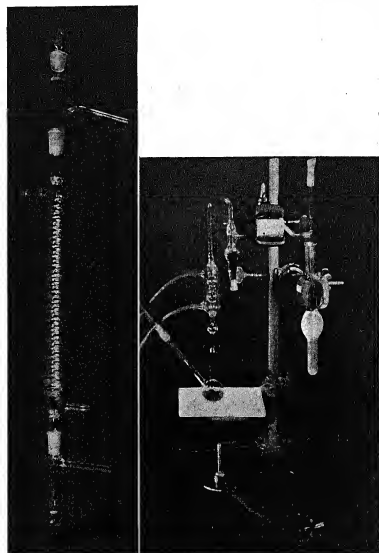


Fig. 1 (left). Ethylene absorption unit. Fig. 2 (right). Modified micro-methoxy apparatus.

chloride; (7) a copper cooling coil; (8) the absorption unit (fig. 1); (9) a freezing trap immersed in a solid carbon dioxide-alcohol bath; (10) a gas-washing bottle containing a 30 per cent potassium hydroxide solution; (11) a gas-washing bottle containing a 5 per cent potassium iodide solution, this vessel being connected to a suction pump. The purpose of the first four units was to insure that the air passing through the balance of the system was free from any trace of ethylene.

Conversion of ethylene dibromide to acetylene was accomplished by heating with alcoholic potassium hydroxide. Absorption of acetylene by various silver solutions was tested, and it was found that alcoholic

silver nitrate gave the best results (Gettler, Niederl, and Benedetti-Pichler, 1932). Ammoniacal silver solutions, as indicated by Weaver (1916), were found to absorb acetylene very slowly, and silver solutions in acetone and water also proved unsatisfactory for the same reason.

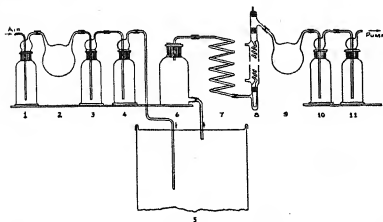


Fig. 3. (1) a gas-washing bottle containing liquid bromine; (2) a freezing trap immersed in a solid carbon dioxide-alcohol bath; (3) a gas-washing bottle containing a 30% potassium hydroxide solution; (4) a gas-washing bottle containing a 5% potassium iodide solution; (5) four 55 gallon drums in parallel each holding 100 pounds of bananas; (6) a large drying bottle filled with calcium chloride; (7) a copper cooling coil; (8) the absorption unit (fig. 1); (9) a freezing trap immersed in a solid carbon dioxide-alcohol bath; (10) a gas-washing bottle containing a 30% potassium hydroxide solution; (11) a gas-washing bottle containing a 5% potassium iodide solution, this vessel being connected to a suction pump.

Apparatus (fig. 2) for this purpose and for the subsequent absorption of the acetylene formed was devised by modification of Pregl's micro-methoxy apparatus (Niederl and Niederl, 1938; Pregl, 1930; Roth and Daw, 1937) so that it was possible to reflux the ethylene dibromide-alcoholic potassium hydroxide mixture. This apparatus consists in its essentials of the bulb, the water-cooled condenser, the gas-washing arrangement, and the capillary outlet, which extends into the receiver. The sample to be tested and five ml. of saturated alcoholic KOH are introduced into the bulb, the gas washer is filled with water, and the receiver is charged with four per cent alcoholic silver nitrate solution (Gettler, Niederl, and Benedetti-Pichler, 1932). After 30 to 40 minutes refluxing on a water bath at 90° to 95°C., the gas is flushed through the system with 200 ml. of air, previously washed with alcoholic silver nitrate (fig. 2), over a period of 30 to 40 minutes into the absorbing tube.

It was recognized that the method outlined above would not serve for accurate quantitative estimation, the most serious obstacle being the incomplete conversion of ethylene dibromide to acetylene. The first product in this reaction is vinyl bromide, which is a gas at room temperature, and even on prolonged refluxing, Bernoulli and Kampli (1933) reported but 57 per cent conversion of ethylene dibromide to acetylene.

Determinations made by the present method on 3-5 mg. samples of pure ethylene dibromide gave recovery varying between 40 and 60 per cent. A few typical results are given in table 1.

For qualitative purposes, approximately 0.1 mg. of silver acetylide is sufficient to produce a very distinct white turbidity, which on agitation by bubbling air through the solution will coagulate and fall to the bottom of the receiver. This bit of material, if isolated, washed, and dried at 100°C., will explode with a distinct pop when heated on a spatula above a flame. This amount of silver acetylide is equivalent to approximately 0.02 ml. of ethylene, allowing for 50 per cent loss from sources indicated in the foregoing.

RESULTS.—The bananas were put in the four drums when they were at the onset of the climacteric. The drums were held at 66° to 68°F., and a continuous flow of air was maintained (30 to 50 l. per hour) sufficient to insure normal ripening. The duration of each run was about seven days, at the end of which time the bananas were fully ripe. Six runs were made, three with and three without bananas in the drums. The order is indicated in table 1. In all cases in which bananas were used the fruit ripened normally, its appearance, taste, and other characteristics being comparable to controls ripened in a regular ripening room.

The material collected in the absorption vessel and the trap was neutralized in all cases with sodium bicarbonate solution to eliminate the excess of the absorbing agent, bromine. The solution was then washed with ethyl ether, in which the olefine bromides are extremely soluble. This ether extract was then distilled until all the ether was removed, and the balance of material was held.

After completing the foregoing work, the extracts from all runs were tested for the presence of ethylene dibromide according to the method previously outlined. All three of the alternate, blank runs, in which the entire apparatus was employed, except that no bananas were used, gave negative results, whereas the other three extracts gave positive results. It will be recalled that this test involved conversion of the olefine bromide derivative into a gas of the acetylene series by treatment with an alcoholic potassium hydroxide solution. The gas thus evolved from the extract of run two was absorbed in cuprous chloride solution, and that from the extract of runs four and six was absorbed in alcoholic silver nitrate solution.

In the first case pink cuprous acetylide was formed, and in the latter two runs, a white precipitate of silver acetylide was obtained. The copper acetylide, after drying, exploded when heated over a flame. Although this is positive evidence that the gas absorbed was either ethylene, propylene, or butylene, all of which have some effect on stimulation of ripening, one more step was necessary to establish beyond question which of the three was given off by the banana. The silver precipitates formed by treatment of these three gases as described above would have silver contents as given in table 1, columns 6 to 8

TABLE 1. Summary of experimental data.

Run	Weight of bananas lbs.	Precipitate	Weight of precipitate mg.	Percentage silver			Ethylene recovery ml./100 lbs. bananas	
				Found	Theoretical		Found	Calculated
					Ag ₂ C ₂	AgC ₂ H ₃	AgC ₂ H ₃	
1	—	—	None	—	—	—	—	—
2	417	Cu ₂ C ₂ b	—	—	—	—	—	—
3	—	—	None	—	—	—	—	—
4	425	Ag ₂ C ₂	4.805	90.22	89.99	73.45	67.06	0.106 0.212 a
5	—	—	None	—	—	—	—	—
6	390	Ag ₂ C ₂	2.643	89.93	89.99	73.45	67.06	0.063 0.126 a
Known ethylene dibromide mg.								
7	4.624	Ag ₂ C ₂	2.531	—	—	—	—	Percentage recovery 42.9
8	3.681	Ag ₂ C ₂	2.802	—	—	—	—	59.6
9	3.317	Ag ₂ C ₂	2.016	—	—	—	—	47.6
Average								50.03%

a Ethylene found multiplied by two, to correct for 50 per cent conversion of C₂H₄Br₂ to Ag₂C₂ (runs 7, 8, and 9).

b Qualitative identification.

The white precipitate obtained as above was analyzed to determine the silver percentage, with the results shown in table 1, column 5.

DISCUSSION.—The silver percentages determined agree so well with the theoretical value for silver acetylide that there can be no question that the precipitates analyzed were derived from ethylene, and no other olefines were present. Calculation from the amount of silver acetylide secured, with allowance for known errors, gives a value of approximately 0.15 ml. of ethylene evolved per 100 pounds of bananas. On this basis it is reasonable to assume that the amount of ethylene liberated is between 0.1 and 0.2 ml. per 100 pounds of bananas.

Gane (1934, 1935) has estimated that the active emanation of apples was of the order of one cubic centimeter per apple (approximately 200 g.) during the whole life of the fruit. Comparison of this value with that obtained for bananas shows that apples evolve more ethylene than do bananas, which confirms the estimates made on the basis of epinastic tests (Denny, 1935). No attempt has been made to determine the mechanism of the action of ethylene in the stimulation of respiratory rate and chemical changes accompanying the acceleration of ripening.

It is, however, safe to state that the confirmation of the evolution of ethylene by fruit tissue reaffirms the belief that this gas has a definite role in the control of plant metabolism.

SUMMARY

Conclusive qualitative evidence of the evolution of ethylene by bananas during ripening was obtained by converting the ethylene to acetylene and determining the latter as silver acetylide.

A method was devised for securing semi-quantitative estimation of ethylene present in small traces in air, and an efficient absorption unit was designed for use in this work.

The amount of ethylene given off by bananas is approximately 0.1 to 0.2 ml. per 100 pounds of fruit during the ripening period.

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ABSORPTION AND TRANSLOCATION OF AUXIN¹

Folke Skoog

TRANSPORT of growth hormone, auxin, formed in the tip of the *Avena coleoptile* was found by Went (1928) to be basipetally polar through sections of this organ. This finding has been confirmed by many

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workers and especially studied by van der Weij (1932, 1934), Pfaeltzer (1934), and Clark (1937). Polar transport of auxin has also been reported to occur in numerous herbaceous plants and some woody plants which have been studied (see Went and Thimann, 1937).

Kögl et al. (1934) have further shown that 3-indole acetic acid, although perhaps not as generally present in higher plants, possesses similar physiological properties. This substance is likewise polarly transported when applied to plants in concentrations within the range of normal physiological activities.

Within recent years the application of 3-indole acetic acid and related compounds in highly concentrated preparations of lanoline paste or aqueous solutions has come into general use. Results from experiments of this type, bearing on the question of transport, have been presented as apparently in contradiction to earlier work.

Thus, Snow (1936) concludes that although normal transport of auxin is basipetal, if higher concentrations are present, transport against the morphological polarity occurs. Laibach and Fischnich (1936a) conclude from experiments on *Coleus* that there is no strict polar transport. Hitchcock and Zimmerman (1935) have concluded that in tomato and tobacco the movement of applied auxin is mainly upward.

It appears then that auxin may be translocated in different directions. Nevertheless, for successful use of applied auxin as a means of promoting growth of plants as well as for an understanding of the apparently contradictory evidence, more direct determinations of absorption and translocation of auxin are essential.

The present work was undertaken primarily for the former purpose, but it bears also on the latter. In this article, therefore, are presented results of experiments in which absorption and translocation of auxin have been studied by quantitative methods in two species.

MATERIALS AND METHODS.—*Solanum lycopersicum*, variety Majestic, and *Cucurbita maxima*, variety Hubbard were chosen as experimental material because they are of suitable size and can be conveniently grown in culture solutions in the greenhouse. The former species was also used by Hitchcock and Zimmerman (1935); the latter species has been extensively used in studies on translocation of electrolytes (Hoagland and Broyer, 1936a); in this laboratory. The experiments were carried out from November, 1936, to April, 1937. The plants were grown in tanks with complete nutrient solutions (Hoagland solution + Supl. 3 A; see Hoagland and Snyder, 1933).² At the time of the experiments they were transferred to individual glass jars containing usually 750 cc. tap water or nutrient solution with and without the addition of given concentrations of freshly prepared solutions of 3-indole acetic acid. The plants were held in corks so that the lowest portions of the stems were never in contact with the external solutions. In all experiments except with very short seedlings the bases of the stems were 3 to 5 cm. above the liquid surface. For a given experiment plants of comparable size and development were selected. Usually both shoots and root systems were maintained intact during the absorption period, but in several experiments to be designated plants were previously decapitated or had 1 to 2 cm. long stretches of stem killed by steam.

Auxin, 3-indole acetic acid (Merck), mostly in the range of concentrations from 0.02 to 2.0 milligrams per liter, was introduced in the following ways: basally, from the solutions in which the roots were kept;

²Supplementary solution modified in H_2BO_3 to 55.0 grams and $ZnSO_4$ to 2.0 grams.

apically, from solutions applied to cut surfaces of stems or to leaves, or from lanoline paste applied to the stems.

At given times, 3 to 4 hours after application of auxin, stem sections 0.5 cm. long were cut at measured distances from the base of the plant, placed on wet filter paper for about 5 minutes, and then on standard size agar blocks for 2 hours. Groups of 2 to 6 sections from a single internode, depending on the size of plants and concentrations present, were placed together on 12 agar blocks. The amounts of auxin obtained were then immediately determined by the standard *Avena* test according to the specifications of Dolk and Thimann (1932) and the modification of 2 decapitations 3 hours apart. Occasionally as a check the sections were placed on a second set of agar blocks for an additional 2-hour period, and the small amounts still remaining were estimated by the deseeded *Avena* test (Skoog, 1937). All operations were carried out on control and treated plants at the same time. The *Avena* tests were done in a dark room at temperature of 24 to 26°C. and relative humidity of 85 to 90 per cent.

Some difficulties not generally met with in work on translocation were encountered. The instability of auxin and the use of the *Avena* test greatly restrict the time allowable for absorption and analyses, respectively. The test further delimits the number of samples that may be used in a single experiment and necessitates use of numerous separate pilot experiments for adjusting concentrations to its quantitative range. In each test about 240 selected *Avena* seedlings were used for analysis of 40 or less samples, including controls. Since results are strictly comparable only within a given experiment, an attempt has been made in presenting the data to include representative examples rather than a compilation of all results. General consideration has been given only to such effects as could be measured in terms of large concentration differences. Concentrations of auxin are expressed in degrees curvature. Under the conditions used 1 degree (1 unit per cc.) corresponds to about 0.005 milligrams per liter freshly prepared 3-indole acetic acid solution. To facilitate interpretation all curvatures indicating presence of auxin (negative) are without sign. The small curvatures (positive) which often appear in absence of auxin are designated as negative.

NORMAL PRODUCTION AND DISTRIBUTION OF AUXIN IN TOMATO AND SQUASH PLANTS.—Terminal buds and stem sections cut from successive internodes of tomato plants at different stages of development, from about 10 to 40 cm. in length, were tested by the standard method of diffusion into agar. Results of only three experiments are included in table 1 (see also fig. 6). The internodes are numbered in order from base to tip. No. 1 represents the internode below the lowest leaf present. They show that the concentration of auxin in terminal buds is relatively high. The concentration in stems is less and decreases from tip to base. From all experiments it may be stated generally that during the early period of growth the

TABLE 1. *Distribution of auxin in tomato plants at different stages of growth. Height of plants, group (a) 7 to 10 cm., (b) 12 to 15 cm., (c) 20 to 25 cm. (No. IV is the most apical internode). Tests done in afternoon. Concentration in degrees curvature.*

Plant no.	a						b					c			
	1	2	3	4	5	Mean	1	2	3	4	Mean	1	2	3	Mean
Internode															
Tip	8	8	11	5	6	7.6	19.5	16.5	15.3	18.0	17.3	25.8	28.3	27.5	27.2
IV							7.8	7.8	8.4	8.0	8.0	10.9	10.2	11.0	10.7
III												6.8	7.4	8.3	7.5
II	3.8	1.9	3.1	4.4	1.1	2.9	6.0	7.8	6.1	7.4	6.8	4.0	3.4	4.7	4.0
I	1.8	1.1	1.0	2.1	1.0	1.4						3.8	2.3	5.5	3.9

hormone concentration increases, but as the plants mature, it gradually decreases. A rough correlation with light conditions from day to day was indicated. In addition there is a diurnal fluctuation in hormone content; considerably higher concentrations are present during the later part of the day than in the morning. These fluctuations are in accordance with the results of van Overbeek on *Raphanus* (1933) and Thimann and Skoog on *Vicia* (1934) showing that auxin is formed only in the light in plants in which storage materials from the seeds have been depleted. The amounts of auxin also vary with individual plants, but this variation is not great enough to interfere with determinations of added auxin when comparable plants are used and tested at the same time.

Squash plants from 40 to 200 cm. in length were tested for auxin in the same manner and gave similar

placing agar blocks in contact with basal surfaces of sections, are in fact evidence of polar auxin transport in both the species used. For a clearer demonstration of polar transport, however, a few experiments of the usual type were done. Sections (0.5 cm. long) cut from successive internodes of tomato plants were placed on wet filter paper for 2.0 hours to eliminate most of the hormone present in them. They were then placed for 2 hours between agar blocks (12 × standard size) one of which contained about 100 units per cc. concentration of 3-indole acetic acid. Alternate sections from the same plants were used for normal and inverse transport and for controls. The amounts of auxin transported through the sections into the plain agar blocks were then immediately determined. Results of one experiment are shown in table 2. It is evident that for the concen-

TABLE 2. *Transport of auxin in stem sections of tomato 0.5 cm. long (from plants 15 to 20 cm. long). Time of transport 20 hours. Concentration of 3-indole acetic acid in applied blocks 100 units per cc.—0.5 milligrams per liter. Each figure is a mean from 6 *Avena* curvatures.*

Plant	1	2	3	4	5	6	7	8	9	10	Mean
a.	17.3	13.0	14.7	15.2	16.8	15.4	16.4	15.2	11.0	18.8	15.4 ± 0.7
b.	13.8	16.5	17.2	—	—	—	—	—	—	—	15.8 ± 1.0
c.	-1.3	-1.0	-1.0	0.3	-0.2	0.6	-0.3	-0.3	-1.2	0.3	-0.4 ± 0.2
d.	-0.5	-0.8	-0.2	0.0	0.2	0.2	2.5	-0.7	-0.7	0.0	0.0 ± 0.3
e.	0.2	0.5	-0.8	-0.5	-0.3	-0.3	-0.5	-0.3	—	—	-0.3 ± 0.1

a. Basipetal transport of applied auxin with gravity.

b. Basipetal transport of applied auxin against gravity.

c. Acropetal transport of applied auxin with gravity.

d. Basipetal transport of auxin present in sections with gravity.

e. Acropetal transport of auxin present in sections with gravity.

results with the exception that smaller amounts were obtained. Concentrations high enough to give significant curvatures in coleoptiles were obtained only from terminal buds and the one to three most apical internodes. Occasionally significant curvatures (up to 5 degrees) were also obtained from lower internodes situated just below developing lateral buds. Squash plants as grown under the above conditions are, therefore, excellent material for determinations of introduced auxin.

POLAR TRANSPORT OF AUXIN THROUGH STEM SECTIONS OF TOMATO.—The foregoing results, obtained by

tration used, auxin transport occurs only in the basipetal direction regardless of whether the sections are so oriented that the direction of transport is with or against the force of gravity.

ABSORPTION OF AUXIN FROM EXTERNAL SOLUTIONS.—Attempts were first made to determine absorption of auxin directly from analyses of external solutions. Jars with and without plants and with and without definite amounts of added auxin were sampled at intervals. The auxin activity of the solutions was found to decrease rapidly but irregularly from one experiment to the next. About half the activity

usually disappeared within 10 hours, and practically all activity was lost within 24 hours after treatment. The rate of decrease was perhaps accelerated by the presence of plants in the solutions but was also so rapid in the absence of plants that an estimation of the amounts absorbed by the plants was not possible by this method. In subsequent experiments, therefore, absorption was determined by the difference in amounts present in control and treated plants.

low values obtained may in part be due to translocation into the stems, to inactivation of auxin within the plants, and to losses in the extraction processes. The possibility of auxin accumulation in the roots from external solutions is not excluded by these experiments.

Translocation into the stem.—In a series of experiments in which the auxin contents of stem sections from control and treated plants were compared at

TABLE 3. Absorption of auxin by roots.

Conc. of external soln.		Time of absorption	Approx. conc. in roots	Method of extraction
Start units/cc.	End			
50	25.5	3.0 hrs.	6.7	Sections placed on agar blocks
0	—		-2.2	
200	127	3.0 hrs.	16.5	Chloroform extraction
75	56	" "	8.7	
0	—	" "	0.0	
100	74	3.5 hrs.	3.2	
0	—	" "	-1.2	

Absorption into roots.—In preliminary experiments auxin was found to be rapidly absorbed into roots from external solutions. Samples of roots of tomato, sunflower, or squash taken after 1 hour or longer periods of absorption were thoroughly rinsed in running distilled water and were then tested either by placing sections on agar blocks or by the chloroform extraction method of Thimann (1934). Samples from treated plants yielded appreciable amounts of auxin. Comparable samples from control plants did not yield detectable amounts by either method. Examples of results of each kind with squash are shown in table 3. The concentrations of recovered auxin were in all cases less than the corresponding initial or final concentrations, per weight, of the external solutions. It was thus not possible to demonstrate an actual accumulation of auxin in roots in the sense used for electrolytes. The methods used, however, were not especially adapted to this purpose. The relatively

different times after addition of 3-indole acetic acid to the external solutions, it was found that auxin absorbed through the roots is under suitable conditions rapidly taken up into the stems. The extent of this upward movement is controlled by several factors, some of which will be considered separately below. Briefly, most efficient uptake into the stems was obtained when the plants in the greenhouse under conditions of rapid transpiration were supplied for periods of about 3 hours with auxin in tap water solutions acidified to pH 4. This arrangement was adopted as a basis for subsequent experiments.

Effect of concentration of auxin.—Uptake of auxin in one experiment from a 100 units per cc. concentration is shown in some detail in table 4. Each figure is the mean value from 5 to 8 *Avena* curvatures. Some similar experiments with different concentrations of auxin are summarized in table 5. The difference in the amounts of auxin present in corres-

TABLE 4. Uptake of auxin into stems (Tomato). Height of plants 7 to 9 cm. Absorption time (a) 4.2 hours; (b) 20 hrs. Solution tap water, pH 4-. Concentration of auxin 0.5 mg. per liter (100 u/cc.).

	Inter-node	Control							Experiment								Uptake
		Plant No.							1	2	3	4	5	6	Mean		
a.	II	3.8	0.0	6.0	5.5	0.0	-0.3	2.5	2.2	3.0	3.0	0.0	3.7	-1.0	1.8	-0.7	
	I	1.1	1.8	1.0	2.1	1.0	-0.5	1.1	15.2	15.6	12.6	16.7	19.4	16.3	16.0	14.9	
	Inverse transport												-1.4	-0.6	-1.0		
b.	II	2.5	0.0					1.3	3.5	-2.0	—				0.8	-0.5	
	I	0.4	-1.4					-0.5	0.9	7.6	10.2				6.2	6.7	
	Inverse transport									-1.0							

TABLE 5. *Uptake of auxin in tomato plants.*

Exp.	Time of absorption	Concentration of auxin in solution	Internode	Control	Auxin treated	Uptake
I	3.5 hrs.	10 units	Tip	35.0	27.5	-7.5
			Internode 2	5.8	3.8	-2.0
			Internode 1	2.6	7.1	4.5
		100 units	Tip	35.0	29.0	-6.0
			Internode 2	5.8	10.2	4.4
			Internode 1	2.6	18.9	16.3
			Inverse transport		0.0	
II	2.5 hrs.	25 units	Tip	8.7	8.7	0.0
			Internode 2	1.7	1.0	-0.7
			Internode 1	1.8	7.0	5.2
III	4.5 hrs.	100 units	Tip	6.4	3.4	-3.0
			Internode 2	2.5	1.8	-0.7
			Internode 1	1.1	17.6	16.5
IV	4.0 hrs.	100 units	Tip	26.5	7.5	-19.-
			Internode 3	6.-	5.-	-1.-
			Internode 2	4.0	12.4	8.4
			Internode 1	2.5	19.1	16.6
		400 units	Internode 3	6.-	7.-	1.-
			Internode 2	4.0	24.8	20.8
			Internode 1	2.5	32.5	30.0
V	4.0 hrs.	25 units	Internode 3	16.0	14.5	-1.5
			Internode 2	16.5	25.6	9.1
			Internode 1	14.4	24.8	10.4
		100 units	Internode 3	16.0	19.3	3.3
			Internode 2	16.5	26.8	10.3
			Internode 1	14.4	31.2	20.8

ponding sections of treated and control plants is taken as a measure of the amounts absorbed. It should be noticed that whereas in control plants the concentration gradient is in general from tip to base of the stems, in treated plants it is reversed (see also fig. 5, 6).

In the experiments listed in table 5 the concentration of absorbed auxin increases with the concentration of the external solution. However, in experiments performed on different days, the amounts taken up from a given concentration differed greatly. This variability may to a very small extent be due to differences in the plants used and to slight differences in the sensitivity of the *Avena* test. Mainly, however, it is due to differences in environmental conditions affecting transpiration during the absorption period, such as light, temperature, and relative humidity, which were not controlled.

The lowest auxin concentration from which measurable uptake into the stem was obtained was 10 units per cc. (0.05 milligrams per liter). Frequently, however, the minimum concentration required was 2 to 3 times greater than this value. In order to determine the relation of the external concentration to the uptake, it is necessary to compare results from different concentrations in a single experiment. Results of a series of such experiments, one of which is pre-

sented in figure 1, show that over a considerable range (at least up to 2 milligrams per liter) the amount taken up is a linear function of the external concentration. Curves I and II represent amounts obtained in the lowest and the second to third from the lowest internodes, respectively. In two experiments in which the concentration range was extended to 800 units per cc. (4.0 mg./l.), the curves gradually flatten near the top. Whether this apparent decrease in efficiency of absorption with higher concentrations is real or is due to limitations of the method of analysis (maximum angles) has not been definitely settled.

Curve III represents results obtained from alternate sections of the same internodes which were placed with their apical ends in contact with the agar blocks. From these results which are typical of all of a large number of determinations (see also fig. 3, curve V), it is clear that although auxin can move upward into the stems of intact plants, in severed sections the movement is polar, occurring only in the basipetal direction even when the sections contain relatively very large concentrations of auxin.

Curves IV and V represent the amounts of auxin obtained in agar blocks placed on basal and apical surfaces, respectively, of alternate sections of petioles from the same plants. It may be seen that in petioles also the movement of auxin is polar. Further-

more, as will be considered later, the amount of auxin in these is very small compared with that in the stem.

Effect of hydrogen-ion concentration.—As mentioned above, absorption of auxin was increased by the acidification of the external solution. Thus from low concentrations measurable absorption would only occur when the solutions were acidified to or below pH 5. Figure 2 represents results of one experiment in which uptake of auxin from two relatively concen-

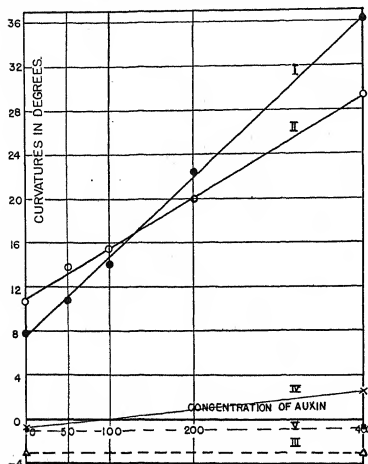


Fig. 1. Effect of concentration of external solution on auxin uptake (Tomato). Height of plant 15 to 20 cm. Absorption time 3.2 hours. Solution tap water, pH 4.2. Conc. in units per cc. Explanation in text.

trated solutions was compared at pH 4.0 and 8.5. Adjustment to the low pH was made by means of a few drops of dilute sulfuric acid; the high pH solutions were plain tap water. It was found unnecessary to buffer the solutions since no change in pH was produced from addition of auxin, and during the short absorption periods used no significant changes in pH occurred. Curves I a and b represent the amounts of auxin obtained from the internodes above the lowest and next lowest leaves, respectively, of plants at pH 4.0. Curves II a and b represent corresponding results from plants at pH 8.5. They show that large amounts of auxin have been absorbed from both the concentrations of external solution at pH 4, whereas no measurable absorption has taken place from the lower concentration at pH 8.5. The amounts taken up from the more concentrated solution might be higher than indicated (maximum angles) in curves I a, I b, and II a, but not in curve II b since the standard deviation from the mean of each point in

the figure is within 1.5 degrees. The amounts of auxin present in control plants was measured only at pH 4, but in similar experiments previously performed no effect of pH on this value was obtained. For example, in one experiment four determinations gave mean values of 5.8 and 6.0 at the two pHs, respectively. It is, therefore, clear that auxin is absorbed more readily from acid solutions, but if the concentration is made high enough, absorption occurs also from slightly basic solutions. In ranges of pH between 5 and 2 good absorption was uniformly obtained, so that it was not possible to ascertain whether an optimum pH exists.

In regard to the mechanism whereby the absorption of 3-indole acetic acid is affected by pH, it should be pointed out that recently Albaum, Kaiser, and Nestler (1937) have determined its penetration into *Nitella* from solutions at different pH values. Their results are in striking agreement with the present ones. They conclude from the effect of pH that 3-indole acetic acid enters the cells in the undissociated form. This interpretation may be correct. Very similar results have also been reported for the absorption of phosphates by van den Honert (1933). Nevertheless, pH may also influence the entry of more neutral salts. Hence suffice it to state here that the effect of pH in the present experiments is probably not to be ascribed to any special properties associated with auxin activity.

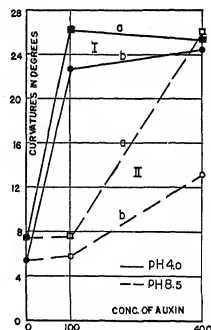


Fig. 2. Effect of hydrogen-ion concentration on auxin uptake (Tomato). Height of plants 15 to 20 cm. Absorption time 3.0 hours. Solution tap water. Concentration in units per cc. Explanation in text.

Effect of concentration of salts.—Preliminary experiments indicated that under identical environmental conditions auxin was more rapidly absorbed from a tap water solution than from the culture solution in which the plants had been grown or from a fresh solution of similar composition. Tests of the activity of auxin added to such solutions in presence of plants showed further that the large decrease in

absorption could not be ascribed to any increase in rate of destruction of auxin.

The effect of salt concentration of the external solution on absorption of auxin was therefore studied. A single salt, KCl, was used. It was found that very dilute solutions would often markedly retard absorption of auxin, but occasionally similar low concentrations distinctly increased absorption. Higher concentrations of salt consistently resulted in decreased absorption. Results of one experiment, in which only the inhibiting effect of salt was obtained, are shown in figure 3. It may be seen that absorption of auxin falls rapidly with increasing KCl concentrations, and at the higher concentrations it is almost completely prevented.

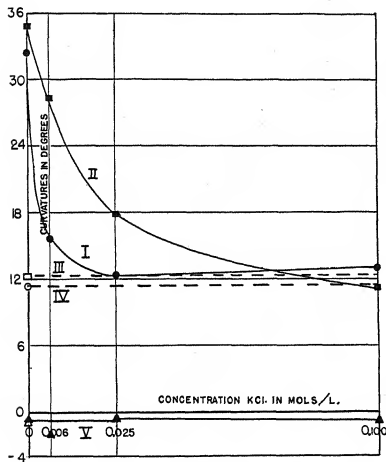


Fig. 3. Effect of KCl on auxin uptake (Tomato). Height of plants 15 to 20 cm. Absorption time 3.0 hours. Solution tap water, pH 4.-. Concentration of auxin 125 units per cc. Curves I and II represent auxin obtained at basal surfaces of sections from lower and middle internodes of plants supplied with auxin. Curves III and IV represent auxin in corresponding control plants. Curve V represents amounts of auxin obtained from apical surfaces of stem sections identical to those of curves I and III.

The relation of salts to the absorption of auxin cannot be satisfactorily explained from present data. Evidently it is not due to competitive absorption of auxin and salt since under certain conditions a distinct increase in auxin absorption is obtained in the presence of salts. It is suggested, and some experiments definitely indicate, that the action of salt is through its effect on the rate of absorption or translocation of water through the plant.

Effect of water movement.—It has been reported by Hitchcock and Zimmerman (1935) that the movement of applied growth-promoting substances, including 3-indole acetic acid, occurs in the vessels and is dependent on the transpiration stream. Experiments below are in agreement with this view in regard to upward translocation through roots from an external solution. In experiments without adequate control or measurement of water movement, it was nevertheless noticed that from external solutions with concentrations of auxin which normally would allow of large amounts of absorption, on rainy or cloudy days little or no uptake was obtained. Furthermore, in a few experiments carried out with squash plants in a large chamber kept at high humidity by a fine spray of water, very slight to no uptake was obtained, whereas in similar plants placed outside the chamber (thus also exposed to light) large amounts of auxin were taken up.

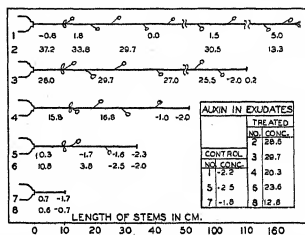


Fig. 4. Effect of transpiration on auxin uptake (Squash). Absorption time 3.1 hours. Solution tap water, pH 4.-. Auxin concentration 800 units per cc. Plants no. 1, 5, and 7 controls; 2, 3, 4, 6, and 8 treated. Numbers represent concentration of auxin in degrees.

In order to show the relation of water movement to auxin uptake, experiments of a different kind were also performed. Squash plants 100 to 180 cm. in length placed in individual jars had different lengths of stem removed so that decreasing numbers of leaves remained (fig. 4). After a three-hour absorption period certain corresponding internodes were analyzed. Since, as may be seen from the figure, the control plants contained very small amounts of auxin, decapitation would not affect the concentration in the stems. Hence, it was not necessary to use separate controls for each treatment. From comparisons of the amounts of auxin obtained from a given internode from the different plants it is evident that practically no auxin reaches near the tip of the internode situated above the highest leaf. The amounts absorbed into lower internodes are in general greater the greater the number of leaves present and also the lower the internode is on the stem. Rough estimates of the loss in volume of the external solution at the end of the absorption period indicated transpirations of about 0 cc. in plants without leaves to about 200 cc. in intact plants.

It was further found that if plants were allowed to absorb auxin and were subsequently decapitated below the cotyledons, large amounts of auxin were present in the xylem exudates (Crafts, 1936) collected from the stumps or obtained by placing agar blocks on the cut surfaces, but tests for auxin in phloem exudates gave negative results. As examples, some results from the plants of figure 4 are included. These were obtained by placing agar blocks for 1 hour on the cut surfaces of stumps cut 6 to 9 cms. below the cotyledons. In general plants which were previously transpiring contained high concentrations of auxin in the exudates, whereas those which had not transpired contained at best very small amounts. Even in plants which were decapitated just below the cotyledons before they were supplied with auxin, relatively high concentrations of auxin could sometimes be obtained by placing agar blocks on the stumps providing that rapid bleeding occurred. In plants which were bleeding very slowly no auxin was obtained at cut surfaces or from apical sections, but sections from the latter taken 5 cm. below the cut surfaces often contained relatively high amounts of absorbed auxin.

There is thus a correlation between absorption of water and auxin absorption both in transpiring and bleeding plants.

Lateral transport and reexport.—The question now arises whether auxin moving with the transpiration stream is carried directly into the leaves and is subsequently reexported or whether a lateral transport occurs in the stems from the vessels into surrounding tissues. Three types of experiments on this point indicate that both alternatives may be operating, but the lateral movement occurring in the stem is perhaps responsible for the main supply of auxin to these tissues. Thus, firstly it was found that, although very high concentrations of auxin may have been absorbed into the stem, petioles of leaves situated along it contain relatively very small amounts of absorbed auxin, and in a number of experiments no absorption could be detected. The results of one such experiment, included as curve IV in figure 1, is representative of the higher values obtained. Secondly, in squash plants supplied with auxin by allowing upper ends of petioles, decapitated just below the lamina or with a small portion of the lamina remaining, to dip into solutions of 3-indole acetic acid, very little to no auxin was found to reach the stems within a three-hour period. Thirdly, the results in the preceding section from plants decapitated below the cotyledons and results in the following section from plants with killed stretches of stem indicate that lateral transport of auxin occurs in the stem.

These results indicate that auxin moving up with the transpiration stream moves laterally into the tissues of the stem and is then reexported by the polar basipetal transport. Lateral movement of auxin has been shown to occur in work on tropisms (Dolk, 1930; van Overbeek, 1933). Polar lateral transport has also been reported for sugars. It has also been considered in movement of electrolytes in roots by

Hoagland and Broyer (1936b). Further, it is clear that upward movement is of a different nature from downward movement. The former is restricted to plants with water moving from the roots, as in these experiments with plants without leaves; or with water moving under tension exerted by transpiration in plants without roots, as in the experiments of Snow and Le Fanu (1936); or a combination of the two, as in intact plants. The latter movement, on the other hand, is restricted by the polarity of the tissues and is independent of water movement.

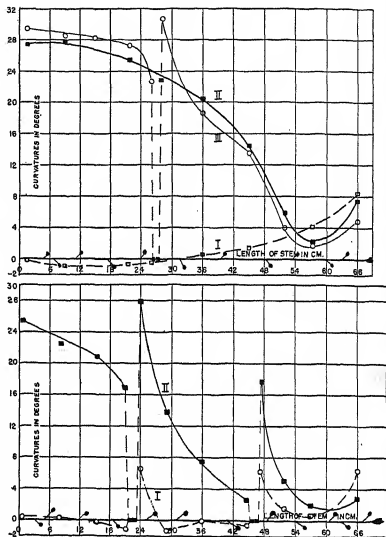


Fig. 5. Distribution of auxin in stems after absorption (Squash). Absorption time 3.0 to 3.5 hours. Solution tap water, pH 4.-. Auxin concentration approximately 400 units per cc.—A (above). Curve I, controls, intact; Curve II, treated, intact; Curve III, treated, one killed section [1.—B (below). Curve I, controls; Curve II, treated. All plants have two killed sections.

PATHS OF TRANSLLOCATION.—*Upward movement.*—It remains to show whether the upward and downward movements occur in different tissues. For this purpose plants from about 50 to 100 cms. in length were used. Some were kept intact and others were ringed in a manner suggested by unpublished work of Hoagland and Broyer (see Crafts, 1936) by killing all living cells in 1 to 1.5 cm. long stretches of internode by application of steam from a fine jet for 10 to 15 minutes. The exposed tissues so treated would dry and shrink so rapidly that only the xylem would

remain open for conduction of materials. From 30 to 60 minutes after steaming the plants were supplied with auxin for 3 to 3.5 hours in different experiments. Since it was necessary to use several treatments, and each with a separate control, only one or two plants of a given treatment could be used in a single experiment. The results of several experiments have therefore been summarized and are expressed in figures 5a and 5b. It was necessary to represent some of the stems as shorter than actual size. In doing so sections at less significant positions, such as near the base, half way between two steamed portions, or near the tip, have been omitted. The characteristic shapes of the curves are kept, but the striking breaks obtained in individual plants are less clearly brought

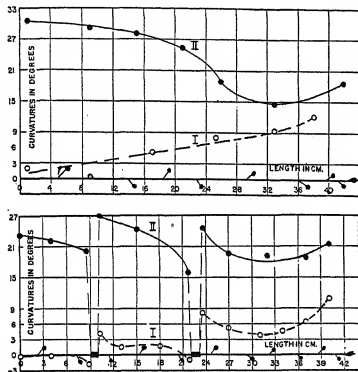


Fig. 6. Distribution of auxin in stems after absorption (Tomato). (Legends same as fig. 5, A and B, respectively.)

out. Sections were removed from the middle portions of the internodes and within 2 to 4 mm. above and below the killed tissues. In figures 5, 6, and 7 each curve represents mean values from 4 or 5 plants.

It may be seen that in control plants auxin does not move across the killed stretches of stem since it accumulates immediately above them and is not obtainable from sections below them. In plants supplied with auxin from the solutions, upward movement is apparently unhindered by the killed sections, but subsequent downward movement of auxin which has passed up is prevented. As a result very high concentrations are accumulated above the rings. These concentrations are even higher than indicated in the figures, since mostly maximum angles were obtained at these points. The results of similar experiments carried out with tomatoes are shown in figure 6.

Downward movement.—In order to compare upward and downward movement of auxin supplied from aqueous solutions, squash plants, which have hollow stems, were supplied with auxin by allowing

the tips of decapitated stems to dip into the solutions. In the three plants so treated large concentrations were found 2 to 3 cm. down the stem, but from this point the concentration gradient decreased rapidly so that practically no auxin could be detected 10 to 15 cm. from the tip. Tomato plants, which have solid stems, were supplied with auxin through glass tubes connected to the cut surfaces of decapitated stems. Results of these experiments are summarized in figure 7. In decapitated but otherwise intact plants (fig. 7a) auxin moves nearly to the base

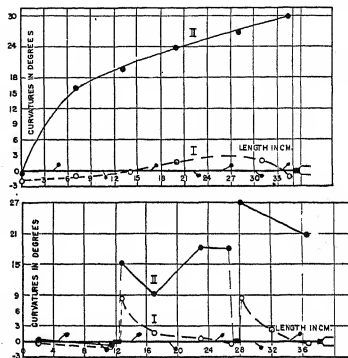


Fig. 7. Distribution of auxin in stems after application of solutions to tips of stems (Tomato). Time of application 3.5 hours. Auxin concentration 500 units per cc. A (above), plants without killed sections; B (below), with two killed sections. Curves I, plants supplied with water; Curves II, plants supplied with auxin solution.

of the stem within three hours. This rapid rate of movement suggests that it occurs in vascular tissues. In plants with a single ring, no transport occurred through the killed tissue, but in plants with two rings (fig. 7b), appreciable amounts of added auxin moved through the upper ring. However, with the exception of a single plant, which was excluded since it was assumed to be imperfectly ringed, no detectable amounts were found below the second ring. It is likely that in these plants auxin solution was actually moving down under tension through the killed tissue of the upper ring to the region between the two rings which was rapidly transpiring. It thus appears that the downward movement of auxin applied in aqueous solutions to plants with adequate water supply from the roots is also mainly in tissues other than the xylem.

This interpretation is supported by the following experiments in which auxin was applied to different portions of the stem in anhydrous lanoline paste. Results of such experiments are shown in figure 8. It may be seen that in these plants movement of

applied auxin is much slower, it occurs only in basipetal direction, and is completely checked by steamed sections. The distribution of auxin in these plants appears in all respects the same as in control plants except that the concentrations are higher. These results are in direct contradiction to those obtained in the same species by Hitchcock and Zimmerman (1935) who concluded that transport of indole acetic acid applied in the form of lanoline paste was not polar. The difference is due to the fact that, by their methods, movement of auxin of the magnitude and kind observed here could not be detected. Possibly if much higher concentrations had been used in the present experiments, leakage into the xylem and upward transport might also have occurred.

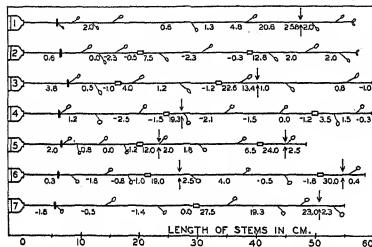


Fig. 8. Distribution of auxin in stems after applications in lanoline paste (Squash). Time of application 5 to 6 hours. 11 Killed sections. ↓ Points of application. Numbers represent concentration of auxin in degrees.

The experiments in this section and especially those obtained from ringed plants demonstrate that upward movement of auxin occurs in the xylem with the transpiration or bleeding stream. Auxin so absorbed moves laterally into the surrounding tissues and is reexported by the normal polar basipetal transport. The downward movement may consist of two parts—namely, from cell to cell in non-vascular tissues and in addition perhaps may occur at a faster rate in the phloem. Under special conditions, in the presence of very high concentrations, auxin introduced into the plant in aqueous solutions may also move downward in the xylem. Downward movement of auxin normally present in the intact plant, however, evidently does not occur in the xylem.

SIGNIFICANCE OF THE RATES OF TRANSPORT.—The rate of translocation of auxin will depend on a number of inter-related external factors and in addition on internal factors connected with the structure and metabolism of the plants.

At present, therefore, only qualitative measurements on the relative rates of upward and downward movements of auxin have been made. Under suitable conditions upward movement in stems of squash occurred over distances of more than 200 cms. within

a three-hour period. These values are lower than the actual rates of transport, since they are measurements in which the total times of absorption and translocation through the root systems and the establishment of large concentrations at the points of analyses on the stems are included. Under conditions of reduced rates of transpiration, upward movement decreased to a point where no uptake into the stems could be detected.

The rate of downward movement of auxin, on the other hand, which is not limited by transpiration may be considered as relatively constant. Including transport in vessels, it was never found to exceed 20 cm. per hour, and in isolated stem sections it is certainly still slower.

A consideration of these facts leads to the following conclusion: auxin will be effectively taken up into growing regions of the stem only when movement through the xylem is at such a rate and concentration that lateral movement into surrounding tissues and subsequent downward transport together with the rate of inactivation are not rapid enough to deplete the transpiration stream of active auxin in the lower portions of the plant.

Hence there must exist for a given set of conditions a rather definite concentration threshold below which no movement of auxin will occur from an external solution to the upper portions of the stem. This concentration will be determined mainly by the rate of absorption and the rate of transpiration. The observed variability in the uptake of auxin from one day to the next can then be readily understood.

Judging from present data, the threshold concentration is usually higher than 0.05 milligrams per liter and can in general not be much lower than this value under conditions in which plants are grown. (The uptake from 0.05 mg./l. conc. was obtained from a more suitable solution than that used for growing plants.) This concentration is also on the lower limit of the range which can be observed to cause stem elongation by direct application.

Nevertheless, it has been found by Hoagland and confirmed by the author (unpublished) that daily application of auxin to culture solutions in concentrations of about 0.00005 milligrams per liter may under special conditions markedly promote the growth of the aerial portions of plants, whereas slightly higher concentrations may not be beneficial. This concentration lies in the range which has been found to stimulate root growth (Thimann, 1937).

Hence it appears from two independent types of evidence—firstly, the range of concentrations necessary for obtaining measurable increase in growth of stems and roots, respectively, and secondly, the concentrations necessary for measurable uptake of auxin from external solutions into the stems—that promotion of growth of aerial portions of plants may be obtained at two different concentration levels. The higher concentration is active after entrance into the tissues concerned, and the lower concentration is active more indirectly through an influence on the

roots. Application in this manner of the higher concentrations is generally not practical for growing plants since it is accompanied by inhibition of root elongation and may have other detrimental effects. Application of the lower concentration, however, will be effective but only under conditions of auxin deficiency.

DISCUSSION.—Results of the experiments have been considered under separate sections. It remains to consider them as a whole and specially in connection with some related problems.

Clearly the present results are in complete agreement with the earlier work on polar transport of auxin. In general they also confirm earlier work on absorption and translocation of applied auxins. However, they extend the latter observations in that more direct, quantitative analyses have been made and lower concentrations within or approximating the range of normal physiological activity have been used. The present experiments further clarify the disagreement in the literature as to transport of auxin, which has arisen as a result of generalizations from experiments of one kind to other kinds done under completely different conditions.

The experiments have been limited largely to auxin in stems and only in this connection have some of the data obtained from roots and leaves been included. It should be pointed out that in the experiments care was taken to keep the root systems intact, so that although occasional plants undoubtedly had a few damaged rootlets, the possibility suggested by Laibach and Fischnich (1936b) that auxin may enter merely through severed roots is excluded.

A possible influence of auxin on polarity in plants referred to by several workers but observed only with extremely high concentrations (Czaja, 1935; Hellings, 1937; Cooper, 1938) does not enter into the present work. Analyses have depended on polar transport, but no significant objections to this procedure are apparent, since low concentrations have been used and only relative amounts of auxin in similar tissues and under comparable conditions have been dealt with. Failure to obtain auxin, as in sections immediately below killed stretches of stem, was not from a lack of polarity in these tissues. The fact that no auxin could be obtained from apical ends of living sections or from either end of the killed sections even when auxin had been passing through them in the xylem indicates that these amounts are either rapidly washed out after cutting or are too small to be detected. The evidence from ringed plants, nevertheless, shows that this fact in no way invalidates the conclusion drawn that in intact plants normal transport of auxin formed in the plant does not occur in the xylem.

Among related problems arising in this work brief attention is given to the following: That under appropriate conditions root formation and development of lateral shoots are controlled by auxin has been established beyond doubt. It is of interest, then, to note that in terms of these functions the distribution of auxin obtained in ringed plants is in complete

agreement with the type of regeneration (briefly, root formation immediately above rings and shoot development below rings) occurring in such plants (Curtis, 1935). An interpretation of correlation and regeneration as controlled exclusively by inorganic nutrients and the carbohydrate nitrogen ratio rather than by a hormone has been given preference by Curtis especially in view of evidence from ringed plants. At present the latter interpretation is equally plausible for this special case and is in fact better supported by direct experimental evidence.

An influence of auxin on absorption and translocation of water and salts noticed in the present work, has been further studied (Skoog, Broyer, and Grossenbacher, 1938). Its bearing on the present work is probably of minor importance and has been neglected.

An effect of introduced auxin on the formation of auxin in the plant has also been observed. These data will be considered elsewhere.

SUMMARY

In the two species investigated, tomato and squash, auxin produced in the plants is transported polarly basipetally through the stems both in intact plants and in cut sections.

This transport may occur both in parenchymatous and vascular tissues but does not normally occur in the xylem.

3-indole acetic acid supplied in external solutions under appropriate conditions is rapidly absorbed through intact roots and translocated into the stem.

This upward movement of auxin occurs with the transpiration stream through the xylem.

Auxin so introduced moves laterally from the xylem into surrounding tissues of the stem and leaves and is then reexported by the normal polar transport.

Various factors such as rate of transpiration, pH, and concentration of salts influence the uptake of auxin from external solutions.

Under given conditions, the uptake of auxin is proportional to the concentration supplied in the external solution over a considerable range of concentrations.

Under conditions of rapid transpiration the rate of upward movement of auxin may be several times faster than the downward movement. The relative rates of the two movements will determine the minimum concentration necessary for uptake into the stem.

Under greenhouse conditions in which plants were grown and experiments performed no absorption of auxin into the stems was obtained from concentrations of less than 0.05 mg. per liter.

The conclusion is drawn that auxin applied in external solutions may influence growth of aerial portions of plants in two ways: (1) High concentrations may be absorbed and act directly on these tissues. (2) Very low concentrations (about 0.00005 mg. per liter) are not absorbed into the aerial portions but may act indirectly through an influence on the roots.

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SELENIUM AS A STIMULATING AND POSSIBLY ESSENTIAL ELEMENT FOR INDICATOR PLANTS¹

Sam F. Trelease and Helen M. Trelease

SELENIUM is of special interest because it is the only mineral element thus far known to be absorbed from the soil by food plants in sufficient quantities to render them lethal to animals (for literature review, see Trelease and Martin, 1936; Beath, 1937; Moxon, 1937). Beath and his associates have found that certain native plants—including several species of *Astragalus* (legume), *Stanleya* (crucifer), *Xylophiza* (composite) and *Onoposis* (composite)—always contain selenium when collected on seleniferous soils (Beath, Eppson, and Gilbert, 1935, 1937; Beath, 1937). These plants frequently accumulate several thousand parts per million of selenium. Furthermore, the evidence obtained by Beath and his coworkers

tends to show that some of these species may be considered as selenium indicator plants, since they apparently occur only on soils that contain selenium. The investigations of Byers (1935, 1936) and Miller and Byers (1937), as well as our own observations in South Dakota, Colorado, Wyoming, Idaho, Nevada, and Utah, support the conclusion that the distribution of Beath's indicator plants is correlated with the presence of selenium in the soil.

Observations in the field therefore suggest that selenium may be an essential element for the growth of the indicator plants. Since the soils supporting these plants contain only traces of selenium, it is apparent that this element, if indispensable, belongs in the group of microtrophic elements, which already

¹ Received for publication February 15, 1938.

TABLE 1. Influence of selenium on dry yields of *Astragalus racemosus* grown in solutions at pH 5.2 with different selenium and sulphur concentrations. Culture period: Oct. 21, 1937, to Jan. 2, 1938.

Cult.	Solution ^a		Dry weight per culture of 5 plants				Ratio to yield of Se-free control ^b	Se content of plants. ppm.
	Se, ppm.	S, ppm.	Grams		Ave.			
	Individual							
1F	0	0	2.04	1.83	2.63	2.17	—	c
2F	"	9	1.12	3.38	2.33	2.28	—	"
3F	"	27	2.17	1.97	1.60	1.91	—	"
4F	"	81	1.80	1.54	3.15	2.16	—	"
5F	"	243	2.57	1.20	1.73	1.83	—	"
6F	"	729	1.60	1.40	1.94	1.65	—	"
7F	"	2187	0.82	0.58	0.79	0.73	—	"
1E	1	0	2.71	2.40	1.99	2.37	1.09	360
2E	"	3	2.71	3.62	3.81	3.38	1.48	258
3E	"	9	4.38	3.21	3.91	3.83	1.68	166
4E	"	27	2.36	3.31	3.00	2.89	1.51	177
1D	3	0	1.47	1.71	2.18	1.79	0.82	609
2D	"	9	2.74	2.91	3.54	3.06	1.34	466
3D	"	27	2.91	3.30	4.48	3.56	1.86	319
4D	"	81	2.84	5.08	2.68	3.53	1.63	419
1C	9	0	1.78	1.59	2.15	1.84	0.85	1413
2C	"	27	3.42	2.72	2.73	2.96	1.55	1221
3C	"	81	5.81	3.70	2.56	4.02	1.86	953
4C	"	243	1.28	2.85	2.46	2.20	1.20	939
1B	27	0	1.51	1.67	0.91	1.36	0.63	3345
2B	"	81	2.15	1.37	1.48	1.67	0.77	1386
3B	"	243	2.39	1.21	4.74	2.78	1.52	1361
4B	"	729	1.89	1.40	2.68	1.99	1.21	1265
1A	81	0	0.28	0.29	0.50	0.36	0.17	3624
2A	"	243	0.63	0.39	0.30	0.44	0.24	3023
3A	"	729	0.21	0.43	0.55	0.40	0.24	2429
4A	"	2187	0.14	0.12	0.17	0.14	0.19	2098

^a All solutions contained: .001 m KH_2PO_4 , .005 m KNO_3 , .002 m $\text{Ca}(\text{NO}_3)_2$, .003 m NH_4NO_3 , .001 m MgCl_2 , .00005 m $\text{Fe}(\text{NO}_3)_3$, .00005 m K-citrate, 15 cc. per liter of S-free microtrophic stock (Trelease and Trelease, 1935). Selenium was added as sodium selenite (Na_2SeO_3) and sulphur as sodium sulphate ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$). With each selenium concentration the S/Se ratios were 0, 3, 9, and 27.

^b The ratios are between yields of Se cultures and Se-free controls receiving the same concentration of S, in ppm.

^c Very low—considered as almost zero.

includes iron, manganese, boron, zinc, copper, and perhaps others.

The experiments reported in the present paper were made in an attempt to obtain direct evidence as to whether or not selenium is capable of stimulating the growth of two of the indicator plants, *Astragalus racemosus* and *Astragalus pattersonii*, when cultivated in artificial media. Following a preliminary test in 1936, the experiments here reported were conducted in the autumn of 1937. A short account of the results has appeared in *Science* (Trelease and Trelease,

1938). We are greatly indebted to Dr. Alan L. Martin for analyzing the plants grown in the solutions.

MATERIALS AND METHODS.—*Astragalus racemosus* was selected for most of the tests. This species occurs widely distributed throughout the Great Plains area, from North Dakota and Wyoming southward to Texas and New Mexico. Seeds (containing 2125 ppm. of selenium) that we had collected about fifteen miles south of Pierre, S. D., were treated with concentrated sulphuric acid for twenty minutes to soften the seed coats and were then soaked in tap water for three

hours. The seeds were germinated in three-inch pots containing quartz sand. After two or three weeks, when the seedlings were about 25 mm. high, they were removed from the sand and mounted in cork stoppers that fitted into one-quart jacketed Mason jars which contained the culture solutions. Each stopper held five seedlings, and the cultures were set up in triplicate. The solutions were renewed at fortnightly intervals during the early growth of the plants and at weekly intervals thereafter.

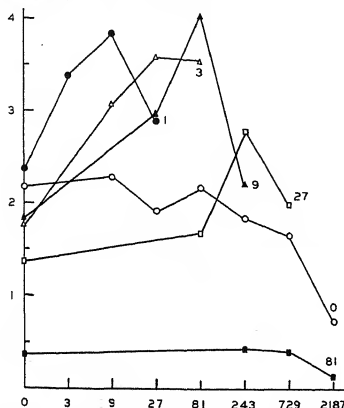


Fig. 1. Growth of *Astragalus racemosus* in solutions with different selenium and sulphur concentrations.

Abscissas represent ppm. of sulphur (log scale); ordinates represent dry yields in grams. Numbers at the ends of the curves denote ppm. of selenium for the series.

One set of cultures was conducted with *Astragalus pattersonii*, using seeds that we collected on a farm in the Grand Valley near Fruita, Colorado.² Analysis of a sample of the plant by Professor Beath showed the presence of 5042 ppm. of selenium. This species is characterized by Wootton and Standley (1915) as malodorous—a property no doubt due in part to volatile selenium compounds like those present in some other species of *Astragalus* and *Oenopsis* which accumulate selenium. *Astragalus pattersonii* is distributed in the drier regions of Colorado, Utah, and New Mexico.

For the sand cultures ten seedlings were planted in three-gallon glazed earthenware jars containing

² This region, which Byers (1935, 1936) had shown to be seleniferous, includes a number of farms purchased by the Federal Resettlement Administration; new houses have been built on them in preparation for leasing to farmers. It is interesting to note that a tract of 100,000 acres of wheat land in South Dakota has recently been acquired by the Resettlement Administration under the marginal land purchase program because of its selenium content and reputation as a toxic area (see Moxon, 1937).

quartz sand and supplied with a continuously renewed culture solution (Trelease and Thomson, 1935).

The cultures were placed in a greenhouse in which they were uniformly exposed to environmental conditions. The daylight period was extended by means of illumination from Mazda lamps (intensity about 250 foot-candles) from 5 p.m. to midnight.

At the conclusion of the culture period the plants in each jar were harvested and weighed, after having been dried for 24 hours at 60°C. The tops of the plants were then pulverized, and 1-gram samples were tested for selenium by a modification of Horn's codeine sulphate method (Martin, 1936).

The results of three series of cultures are presented in this paper: Series 1 comprised solution cultures of *Astragalus racemosus* with different ratios of sulphur to selenium; series 2 was designed to test the influence of various pH values; and series 3 was conducted in order to determine the response of another species, *Astragalus pattersonii*, to selenium. In addition, reference is made to a set of sand cultures that has given results agreeing with those from the solution cultures.

It should be emphasized that the results reported in the present paper are considered to be applicable only to the particular inorganic compounds of selenium used (chiefly selenite). The work of Beath, Eppson, and Gilbert (1937) indicates that our results might have been different if we had used organic forms of selenium, as obtained in water extracts of seleniferous plants.

STIMULATION OF GROWTH BY SELENIUM.—Although the initial concentration of selenium in the seeds of *Astragalus racemosus* was about 2125 ppm., the seedlings which were given no additional selenium made slow growth in comparison with those which obtained this element (as selenite) from the culture solution. Marked stunting of the plants deprived of selenium became evident within about three weeks after their transference to the mineral solution. At the end of a growth period of about two and one-half months the dry yields of the plants that received selenium from the culture solution were considerably greater than those of the plants in the control solutions (tables 1-3 and fig. 1, 2). In a number of cases the absorption of selenium nearly doubled the amount of growth made by the plants. These experiments therefore show that selenium has a pronounced stimulating effect on the growth of *Astragalus racemosus*.

Selenium in concentration from 1 to 27 ppm. stimulated growth when the culture solution contained sulphur (fig. 1). With each concentration of selenium the dry yields of the plants were increased as sulphur (in the form of sulphate) was added. Maximum growth, and hence greatest stimulation, was obtained when the S/Se ratio had a value of 9/1. With this ratio 1, 3, and 9 ppm. of selenium were about equally effective in increasing the yields over those obtained with the control. A concentration of 27 ppm. gave less stimulation, and 81 ppm. was markedly toxic.

TABLE 2. Influence of selenium on dry yields of *Astragalus racemosus* grown in solutions with different initial pH values. Culture period: Oct. 24, 1937, to Jan. 12, 1938.

Initial pH value of solution ^a	Dry weight per culture of 5 plants				Se content of plants, ppm.
	0 Se		9 ppm. Se		
	Grams	Relative	Grams	Relative	
pH 5.2	1.76		2.76		
	2.28		3.48		
	1.12		4.17		
	(0.84)				
pH 5.5	1.72	1.00	3.47	2.02	2464
	2.25		2.62		
	1.87		3.81		
	2.59		4.59		
	2.56				
	2.32	1.00	3.67	1.58	1805
pH 5.8	2.44		4.83		
	3.44		3.51		
	1.99		3.66		
	2.00				
pH 6.4	2.47	1.00	4.00	1.62	1166
	3.52		5.04		
	2.77		4.42		
	2.68		4.13		
pH 7.1	2.99	1.00	4.53	1.52	1795
	(0.77)		4.73		
	3.65		2.30		
	1.67		4.74		
	2.66	1.00	3.92	1.47	1620

^a All solutions contained: .001 m KH_2PO_4 + K_2HPO_4 (to give desired pH), .005 m KNO_3 , .002 m $\text{Ca}(\text{NO}_3)_2$, .003 m NH_4NO_3 , .001 m MgSO_4 , .00005 m FeSO_4 , .00005 m K-citrate, 15 cc. per liter of microtrophic stock (Trelease and Trelease, 1935). Selenium was added as sodium selenite (Na_2SeO_3). The S/Se ratio (ppm.) was 3.8/1. For pH 5.2 100% of the phosphate was KH_2PO_4 ; for pH 5.5, 95%; for pH 5.8, 84%; for pH 6.4, 65%; and for pH 7.1, 0%.

It may be seen from table 2 that greatest growth of *Astragalus racemosus*, both with and without selenium, occurred in the culture solutions having an initial pH value of 6.4. (Final values, before solution renewal, rarely differed from the initial values by more than 0.5 pH.) Improvement in growth due to the addition of selenium appears to have been about the same at the various pH values.

Stimulation of *Astragalus pattersonii* is shown by the data of table 3. This species, though very different from *Astragalus racemosus* in growth habit, responded similarly. Moreover, sodium selenite and potassium selenate were about equally effective as sources of selenium.

The results obtained with solution cultures have been confirmed with sand cultures (fig. 2), not yet harvested. These have received continuously renewed solutions at pH 6.0. Best growth has been made with 9 ppm. of selenium (as sodium selenite),

but growth with 1 and 3 ppm. is nearly as good, and exceeds that of the control. The plants grow more vigorously in sand cultures than in solution cultures; greater aeration in the sand probably keeps the roots in a healthier condition.

After we had completed one set of experiments on the growth of *Astragalus racemosus* in artificial media, we learned from Professor Beath that he had obtained similar results with soil tests. Germination and growth of *Astragalus pectinatus* and *Astragalus bisulcatus* were markedly stimulated when a soil low in naturally occurring selenium was treated with a solution carrying 40 ppm. of selenium (as sodium selenite). It was found to be impossible to secure a stand of either of these species of *Astragalus* on a Cecil clay loam from North Carolina.

Levine (1925) reported stimulated growth of white lupine and timothy seedlings in solutions of 0.0001 per cent selenium dioxide and 0.001 per cent selenic acid.

TABLE 3. Influence of selenium on dry yields of *Astragalus pattersonii*. Culture period: December 5, 1937, to February 1, 1938.

Treatment	Dry weight per culture of 5 plants ^a		Se content of plants, ppm.
	Grams	Relative	
Solution 1 ^b			
0 Se	1.06	1.00	0
9 ppm. Se (Na ₂ SeO ₄)	1.68	1.58	484
18 ppm. Se (Na ₂ SeO ₄)	1.38	1.30	698
9 ppm. Se (K ₂ SeO ₄)	1.60	1.51	838
18 ppm. Se (K ₂ SeO ₄)	1.42	1.34	923
Solution 2 ^c			
0 Se	1.12	1.00	0
9 ppm. Se (Na ₂ SeO ₄)	1.38	1.23	1313
9 ppm. Se (K ₂ SeO ₄)	1.37	1.22	1358
18 ppm. Se (K ₂ SeO ₄)	1.56	1.39	2110

^a Ave. based on 3 cultures.

^b Same solution as used for pH 6.4 in the hydrogen-ion series.

^c Solution 1 modified as follows: phosphates, calcium nitrate, and magnesium sulphate, $\times 2$; ammonium nitrate, $\times 0.67$; potassium nitrate omitted.

Stoklasa (1922) noted a beneficial influence of selenates in low concentration. Martin (1936), however, obtained only toxic effects of selenite on the growth of wheat and buckwheat. Hurd-Karrer (1937) found that 2-4 ppm. of selenium (as sodium selenate or sodium selenite) stimulated wheat seedlings grown for

five weeks in nutrient solutions. Since Hurd-Karrer's maximum stimulation of wheat was only 13 per cent, it was not comparable to the increase of more than 80 per cent obtained in our experiments with *Astragalus*. Beath, Eppson, and Gilbert (1937) observed that in some cases cereals and forages were stimulated by organic compounds from seleniferous wild plants, but they considered that this might have been due to other factors than selenium activation.

The pronounced stimulating effect of selenium on the growth of *Astragalus racemosus* and *Astragalus pattersonii* suggests that this element may be essential for the development of various species of indicator plants. In several cases—e.g., with boron, manganese, and zinc—a mineral element regarded at first as merely stimulating has later been shown to be indispensable for plant growth. Selenium, if required by certain plants, appears to be unique among the essential elements in being needed by only a few species of the higher plants, in the Leguminosae, the Compositae, and the Cruciferae. Even in the genus *Astragalus* some species appear to be definite indicators, limited to seleniferous soils, while others are indifferent in their soil requirements (Beath, 1937; Beath, Eppson, and Gilbert, 1935, 1937). These observations suggest an interesting evolutionary development of requirement of selenium by a small number of species belonging to distantly related families.

The usefulness of indicator plants in the mapping of seleniferous areas is obvious. If certain plants can be shown to require selenium for their growth and never to occur on soils which lack this element (or on soils in which its concentration falls below a certain minimum), then the mere presence of these plants would indicate seleniferous soils. Any plant, however, which is capable of accumulating selenium—even though it does not require this element—is an

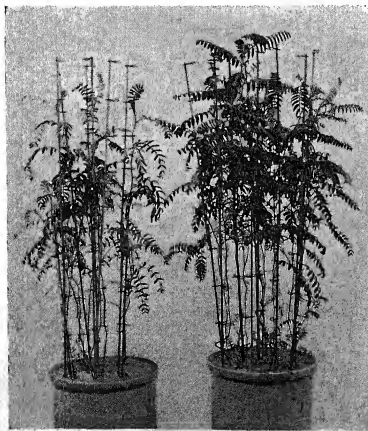


Fig. 2. Stimulating influence of selenium on *Astragalus racemosus*. The plants on the left received no selenium; those on the right received 9 ppm. of selenium (as selenite) in the culture solution. The plants were grown from seed for five months in 3-gallon jars containing quartz sand.

aid in locating seleniferous regions, because the high concentration in the plant is much more easily detected by chemical analysis than is the low concentration in the soil.

TOXICITY OF SELENIUM.—Since plants of *Astragalus racemosus* are stimulated by selenium and sometimes accumulate as much as 15,000 ppm. without visible injury (Beath, 1937; Beath, Eppson, and Gilbert, 1935, 1937), it is perhaps not surprising to find that they can tolerate a relatively high concentration of selenium in the culture solution. With 81 ppm. of selenium (as sodium selenite) the *Astragalus* plants were stunted and chlorotic, exhibiting symptoms similar to those of wheat and buckwheat plants in a solution containing 8 ppm. (Martin, 1936). From figure 1 it may be seen that with the two highest S/Se ratios 27 ppm. of selenium stimulated the growth of the plant as a whole (though retarding root development), whereas Martin found that even with high S/Se ratios 2 ppm. was markedly toxic to buckwheat and 4 ppm. produced similar effects in wheat. Thus *Astragalus racemosus* is capable of tolerating approximately ten times the concentration of selenium (as selenite) that can be withstood by wheat and buckwheat.

An antagonistic influence of sulphur on the toxicity of selenium is evident in figure 1 for the solutions containing 27 ppm. of selenium. With S/Se ratios of 0 and 3 the dry yields are below those of the controls, but with ratios of 9 and 27 they exceed those of the controls. It may be noted, however, that the addition of sulphur had little effect in overcoming the toxicity of 81 ppm.

That germinating seedlings of *Astragalus racemosus* tolerate higher concentrations than older plants is shown by the data of table 4 on growth during the 4-day period following germination. About 256 ppm. was required to reduce the root growth of the seedlings to 1/5, whereas 81 ppm. was sufficient to bring about a similar reduction in the growth of older plants (table 1). The *Astragalus* seedlings tolerated about eight times as high a concentration as germinating wheat seedlings (Martin, 1936).

It should be emphasized that symptoms of selenium injury of plants have never been reported from observations in the field. Neither crop plants nor native range plants show any signs of stunting or chlorosis when growing on naturally occurring seleniferous soils (Beath, Eppson, and Gilbert, 1937). The selenium problem has not been shown to involve diminished yields in pasture or in crops, but is concerned with poisoning of livestock and possible injury to human beings from the consumption of seleniferous grains, vegetables, and animal products. Extensive investigations into the poisoning of animals have already been made. The research required on human phases of the problem seems barely to have been touched. It may be noted, however, that Smith, Franke, and Westfall (1936) found that among 127 specimens of urine representing subjects from 90 families in seleniferous areas of South Dakota, Wyoming, and Nebraska,

TABLE 4. Growth of germinating seedlings of *Astragalus racemosus* during 4-day period in the dark at 29°C. Initial root length 10 mm.; hypocotyl length, 2 mm. Selenium added as sodium selenite to culture solution given in table 1. Each mean is based on measurements of 25 seedlings.

Solution		Mean elongation, mm.		
Se, ppm.	S, ppm.	Root	Hypocotyl	Total
0	0	28	25	53
"	40	26	26	52
"	80	26	23	49
"	160	31	26	57
"	320	28	25	53
"	640	30	24	54
"	1280	31	21	52
8	0	30	26	56
"	40	31	29	60
"	80	32	24	56
"	160	31	26	57
16	0	26	24	50
"	80	36	25	61
"	160	35	25	60
"	320	33	26	59
32	0	24	26	50
"	160	31	24	55
"	320	31	25	56
"	640	34	24	58
64	0	19	26	45
"	320	23	24	47
"	640	22	24	46
"	1280	23	21	44
128	0	13	22	35
"	640	12	18	30
256	0	7	17	24
"	1280	3	10	13
512	0	1	9	10
"	2560	0	5	5

92 per cent were positive for selenium (0.02 to 1.33 ppm.).

SELENIUM ACCUMULATION.—The presence of organic selenium compounds in all the plants except the controls was indicated by their extremely offensive garlicy odor while being dried and ground for analysis. The odor was strongest in the plants grown in the most concentrated selenium solutions, and it was not perceptible in the control plants. The intensity of the odor gives some idea of the selenium content of certain species of *Astragalus*, as was pointed out in 1934 by Beath, Draize, Eppson, Gilbert, and McCreary. In the field the odor of plants of *Astragalus* and *Oenopsis* which accumulate selenium is so strong that it frequently betrays their presence to one traveling in a rapidly moving automobile.

It may be noted from figure 3 (plotted from the data of table 1) that the accumulation of selenium by *Astragalus racemosus* growing in solution cultures was directly related to the concentration of selenium (as selenite) and inversely related to that of sulphur (as sulphate). In the absence of sulphur, increasing the selenium concentration of the culture solution from 1 to 81 ppm. brought about an increase in the selenium content of the plants from 300 to 3624 ppm. The maximum accumulation by *Astragalus* was twenty times the amount stored by soy bean plants growing in sand cultures (Martin and Trelease, 1938). Some loss undoubtedly occurred during the process of drying (Beath, Eppson, and Gilbert, 1935), which was considered necessary for obtaining reliable growth data.

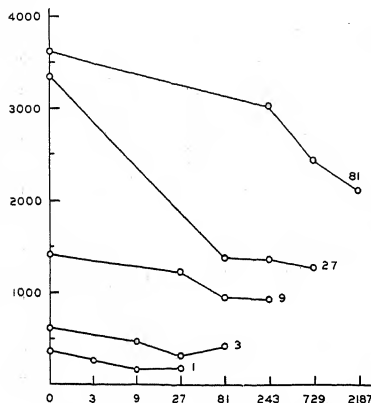


Fig. 3. Accumulation of selenium by *Astragalus racemosus* grown in solutions with different selenium and sulphur concentrations.

Abscissas represent ppm. of sulphur (log scale); ordinates represent selenium content of the plants in ppm. Numbers at the ends of the curves denote ppm. of selenium in the culture solution.

By using culture solutions with a S/Se ratio of 27/1, the selenium content of the *Astragalus* plants was reduced to from 38 to 66 per cent of the amount present in the plants without sulphur (fig. 3). Hurd-Karrer (1935) and Hurd-Karrer and Kennedy (1936) reported similar or even greater reduction in wheat. Even with an excess of sulphur in the solution the selenium content of the *Astragalus* plants ranged from 177 ppm. (absorbed from a solution with 1 ppm.) to 2098 ppm. (from a solution containing 81 ppm.). Thus sulphur was not very effective in checking the absorption of selenium. These results indicate that sulphur is no more effective in preventing the absorption of selenium by *Astragalus race-*

mosus and other native range plants than it is in inhibiting the absorption of selenium by wheat and corn (Franke and Painter, 1937) or by tobacco and soy beans (Martin and Trelease, 1938). Beath, Eppson, and Gilbert (1937) found that sulphur and soluble sulphates added to various soils had no antagonistic effect on selenium absorption by wheat, beans, and other food plants when the source of available selenium was supplied by powdered seleniferous weeds; on the contrary, the sulphur had a tendency to increase the selenium intake rather than to retard it. Most of the naturally occurring seleniferous soils are already saturated with sulphur in the form of gypsum. Beath (1937) refers in particular to one of the most toxic areas in eastern Wyoming, which is located on residual Pierre soil. The surface foot of soil carried 0.67 per cent of soluble sulphates and 8.6 ppm. of selenium; the second foot, 1.16 per cent of soluble sulphates and 22.7 ppm. of selenium. *Astragalus racemosus* growing in this highly sulphurized soil contained 14,920 ppm. of selenium. According to Beath, this and numerous other toxic areas, regardless of their high sulphur content, support not only native seleniferous weeds but also toxic farm crops. Thus there seems to be abundant evidence to support the conclusion that addition of sulphur to the soil would not be a practicable means of preventing native range plants or farm crops from becoming poisonous through the absorption of selenium.

Low concentrations of selenium in food are capable of producing fatal injury to mammals. Many cases have been reported of the death of horses, hogs, cattle, and sheep that have consumed naturally toxic grains and fodder with selenium contents indicated as somewhat less than 50 ppm. When rats were fed on a diet containing 65 ppm. of selenium derived from ground pods and seeds of *Astragalus racemosus*, they were killed within 4 to 11 days (Trelease and Trelease, 1937). Franke (1934) found that grain containing about 30 ppm. of selenium, obtained from farmers whose livestock had been affected, killed 92 per cent of the rats within 100 days. Martin's (1936) experiments showed that when 22 ppm. of selenium was present in the diet (either as sodium selenite or as selenium which had been absorbed from the soil by buckwheat plants), death of most of the rats occurred within 42 days. Similar food containing 64 ppm. of selenium was lethal to all rats within 7 days. Munsell and DeVany (1936) reported that a diet containing only 6 ppm. of selenium, derived from toxic wheat, had a detrimental effect on the growth and reproduction of rats. Certain insects, however, exhibit a remarkable tolerance to selenium (Trelease and Trelease, 1937). It was found that bruchids (beetles of the species *Acanthoscelides fraterculus*) and seed-chalcids (hymenopterous insects, *Bruchophagus mexicanus*) could consume seeds of *Astragalus bisulcatus* containing 1475 ppm. of selenium and complete their life cycle.³

³ In the present experiments it was necessary to fumigate several times with cyanide to destroy white flies

The selenium content of the *Astragalus* cultures that made greatest growth is shown in table 5, which summarizes the data given in table 1. Although the yields were nearly the same with 1, 3, and 9 ppm. of selenium in the culture solution, the amount of selenium in the plants ranged from 166 to 953 ppm. The minimum selenium content for high yields was therefore not more than 166 ppm., which the plants could accumulate from a solution containing only 1 ppm.

TABLE 5. Selenium content and growth of *Astragalus racemosus*. Data from table 1.

Selenium concentration of solution, ppm.	Selenium content of plants, ppm.	Dry yields, grams
1	166	3.83
3	319	3.56
9	953	4.02
27	1361	2.78

Although a marked reduction of growth was associated in these experiments with a selenium content greater than 2000 ppm., Beath, Eppson, and Gilbert (1937) observed no toxic symptoms in *Astragalus racemosus* which was growing in the field and contained 15,000 ppm. of selenium. These workers have shown that the organic forms of selenium, as obtained in water extracts of seleniferous plants, are much more available and less toxic to plants than the inorganic compound of selenium (selenite) used in the present tests.

The tolerance of some insects, such as bruchids and seed-chalcids, to high concentrations of selenium in their food apparently depends upon a failure of these that attacked *Astragalus racemosus* in one set of sand cultures receiving 0, 1, 3, 9, and 27 ppm. of selenium (as sodium selenite). And on April 22, 1938, all the plants in this set became heavily infected with powdery mildew, presumably *Erysiphe polygoni* DC. from beans. It is evident that these parasites were not adversely affected by high concentrations of selenium in the plants.

insects to absorb selenium (Trelease and Trelease, 1937). In plants, however, it may be supposed that tolerance involves a conversion of the selenium into organic compounds which are non-toxic to the living cells—selenium being stored as a component part of the proteins of grains (Franke and Painter, 1936) or present in water-soluble substances in the native selenium accumulators, such as *Astragalus racemosus* (Beath, Eppson, and Gilbert, 1937).

SUMMARY

Selenium (as selenite) in concentrations of from 1 to 27 ppm. had a pronounced stimulating effect on the growth of *Astragalus racemosus* in solution cultures and sand cultures. *Astragalus patersonii* responded similarly. These experiments, which supplement field observations on the distribution of the plants, suggest that selenium may be an essential microtrophic element for these and other indicator plants, which include several species of *Astragalus*, *Stanleya*, *Xylorhiza*, *Oenopsis*, etc. The usefulness of indicator plants in locating and mapping seleniferous areas is obvious.

Astragalus racemosus tolerated about ten times the concentration of selenite that can be withstood by wheat and buckwheat. Sulphur (as sulphate) tended to reduce the toxicity to the plants of the lower concentrations of selenium.

The accumulation of selenium by *Astragalus racemosus* was directly related to the concentration of selenium and inversely related to that of sulphur in the culture solution. An excess of sulphur, though reducing the selenium content to between one-third and two-thirds of the amount present without sulphur, did not prevent these native range plants from accumulating enough selenium (177 to 2098 ppm.) to render them potentially lethal to animals.

The results reported in this paper are considered to be applicable only to the particular inorganic compound of selenium (selenite) used in the culture solution.

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ABSORPTION OF SELENIUM BY TOBACCO AND SOY BEANS IN SAND CULTURES¹

Alan L. Martin and Sam F. Trelease

IT HAS been shown by several authors that certain crop plants vary greatly in their ability to withstand high concentrations of selenium and in their tendency to accumulate the element (Byers, 1935; Beath, Eppson, and Gilbert, 1935; Hurd-Karrer, 1935; Martin, 1936; Trelease and Martin, 1936). Since much of the seleniferous territory in this country is used for raising food plants or supplying forage for cattle, the selenium problem has important relations to public health. (For reviews of the literature, see Trelease and Martin, 1936; Beath, 1937; Moxon, 1937.) Sulfur has been reported as partially counteracting the toxic effects of moderate concentrations of selenium to certain plants (Hurd-Karrer, 1933, 1934; Martin, 1936) and as substantially reducing the amount stored in the tissues (Hurd-Karrer and Kennedy, 1936).

The present investigation was undertaken, during the winter and spring of 1937, in order to compare selenium toxicity in tobacco and soy beans and to determine the effectiveness of sulfur in checking the injury and in reducing the accumulation of the poison by the plants.

EXPERIMENTS WITH TOBACCO.—Turkish tobacco seeds were germinated and grown for two weeks in flats containing quartz sand, where they were watered daily with a balanced nutrient solution. At the end of a month, when the plants were 2 cm. high and bore 5 leaves, they were transplanted to 6-inch paraffined pots filled with quartz sand. The plants were grown on rotating tables in the greenhouse and were

supplied with continuously renewed nutrient solutions (Trelease and Thomson, 1935). All solutions contained the following salts: ammonium nitrate, 0.001 m; potassium dihydrogen phosphate, 0.001 m; calcium nitrate, 0.002 m; magnesium chloride, 0.001 m; ferric nitrate (with an equal amount of potassium citrate), 0.00005 m. In addition, the microtrophic elements were supplied in the concentrations suggested by Trelease and Trelease (1935), but with all sulfur compounds replaced by chlorides. Five series of cultures were set up, adding selenium as sodium selenite (Na_2SeO_3) to provide concentrations of 1, 2, 4, 8, and 16 ppm. The conclusions which are drawn are considered as applicable only to the particular form of inorganic selenium used in these experiments. The results might have been different if selenates (Hurd-Karrer, 1937) or water-soluble organic compounds of selenium (Beath, Eppson, and Gilbert, 1937) had been used.

For determining antagonism, sulfur, as sodium sulfate ($\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$), was supplied in each series so as to give ratios (in ppm.) of sulfur to selenium of 2.5:1, 5:1, 10:1, and 20:1. Sets of control plants received solutions containing the various concentrations of sulfur but lacking the selenium. All cultures were set up in duplicate. Notes on the appearance of the plants were recorded weekly, and final dry weights were obtained at the end of 64 days.

Injury and evidence of toxicity.—Symptoms of selenium toxicity were not so clearly defined or consistent for tobacco as for wheat and buckwheat. (Martin,

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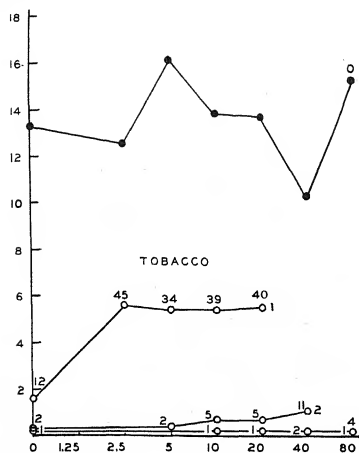


Fig. 1 (left). Mean weights (in grams) of the tops of tobacco plants. Each mean is from two plants grown in sand cultures in the greenhouse for 64 days.

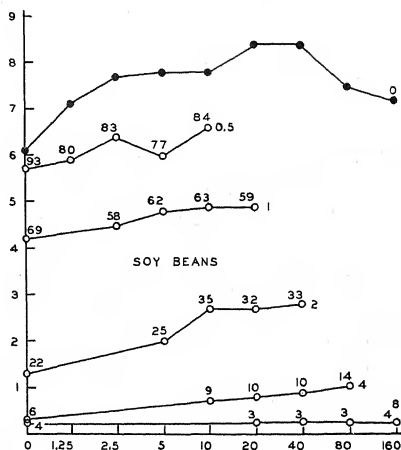


Fig. 2 (right). Mean weights (in grams) of tops of soy bean plants. Each mean is from 12 plants grown in sand cultures in the greenhouse for 56 days.

Abscissas represent ppm. of sulfur (as sulfate), ordinates represent growth (dry weight in grams). Numbers at the ends of the curves indicate ppm. of selenium (as selenite) in the series. Numbers near the points on the curves show percentages of the values for the selenium-free cultures.

1936). In wheat the destruction of the chlorophyll resulted in some "snow-white" leaves; typical chlorosis of buckwheat leaves started near the edges and progressed inward until all intervenous regions were ivory-white, the veins remaining green. In the less severely injured tobacco plants, either a yellowish chlorosis developed in the lower leaves while the growing tips remained dark green and turgid, or the reverse order of tissue injury occurred. The number and size of leaves on the injured plants, however, were reduced in all cases. The smaller leaves were puckered and excessively hairy. They remained turgid and dark green until they began to wither around the edges. Stunting due to selenium toxicity was common to tobacco, wheat, and buckwheat, but occurred at lower concentrations in tobacco than in the other plants. A concentration of 4 or more ppm. of selenium in the culture solution prevented stem elongation of tobacco plants.

Toxicity in the absence of sulfur.—The four lower leaves of the plants given 1 ppm. of selenium without sulfur died earlier than those of the selenium-free controls. At the time of harvesting, the injured plants averaged 15 chlorotic leaves and 11 leaves with normal green color. The control plants had 49 leaves. Stunting due to this concentration of selenium reduced the dry weights to 12 per cent of those attained by the control plants.

By the seventh day the first 3 leaves were wilted on the plants receiving 2 ppm. of selenium without sulfur. The leaf count at harvesting averaged 5 dead, 5 chlorotic, and 10 with an abnormally dark-green color. The outer margins of the leaves frequently dried up, while the living regions were chlorotic but had dark green veins. The dry weights were reduced to 2 per cent of those for the controls.

The young leaves which developed on the plants given 4 ppm. of selenium without sulfur were very turgid, puckered, and dark green. At the time of harvesting, the first 5 leaves were dead, the next 2 were chlorotic, and the remaining 4 were turgid and dark green. Although new leaves had developed, the stems had not elongated.

The application of 8 ppm. of selenium without sulfur killed all the plants by the thirty-sixth day. With 16 ppm. of selenium all but 2 leaves were wilted on the second day after the experiment was started; the plants were dead by the thirty-third day.

Low concentrations of selenium injured tobacco more seriously than buckwheat, wheat, or soy beans. Although solution cultures were used in the earlier work (Martin, 1936) and sand cultures were employed in the experiments with tobacco and soy beans, similar solutions were used in both cases. Tobacco was killed in thirty-six days by 8 ppm. of selenium, but wheat and buckwheat in this concentration of

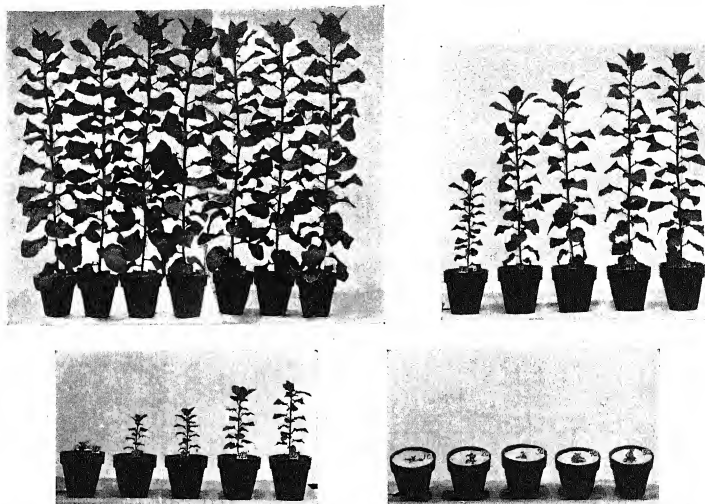


Fig. 3. The toxicity of selenium to tobacco plants grown in the greenhouse for 64 days. Series F (upper left) had no selenium, but the sulfur content varied from 0 to 80 ppm. Series E (upper right) received 1 ppm. of selenium; series D (lower left), 2 ppm.; series C (lower right), 4 ppm. The culture at the extreme left in each series had no sulfur. The sulfur: selenium ratio in the other cultures increased from left to right—2.5:1, 5:1, 10:1, 20:1.

selenium remained alive for forty-two days, and soy beans for fifty-six days.

Effect of sulfur on toxicity.—Sulfur has been previously reported as effective in partially counteracting selenium toxicity to wheat (Hurd-Karrer, 1933, 1934, 1935, 1937; Martin, 1936) and buckwheat (Martin, 1936). Since the degree of antagonism between these two elements differed somewhat with the species of plants, it was of interest to see how tobacco would respond.

All the plants supplied with solutions containing selenium showed distinct evidence of injury regardless of the amount of accompanying sulfur. The photographs taken prior to harvesting (fig. 3) show that stunting due to 1 ppm. of selenium was partially counteracted by sulfur. The dry weights of the plants receiving 1 ppm. of selenium were increased by the addition of sulfur (fig. 1). Considerable antagonism is evident, since sulfur raised the dry weights from 12 per cent to from 34 to 45 per cent of those of the controls. Antagonism was considered to have existed whenever the addition of a given concentration of sulfur to a solution containing selenium produced greater increase in growth than was produced by the addition of the same concentration of sulfur to a selenium-free solution. The greatest antagonism with 1 ppm. of selenium occurred with the first addition

of sulfur. Although the final weights were about the same for each subsequent increment in the sulfur concentration, the percentage in relation to the sulfur controls decreased.

With each increase in the sulfur:selenium ratio, plants in 2 ppm. of selenium produced more growth. The greatest weight attained, 11 per cent of that for the corresponding control, was with a 20:1 ratio.

There was practically no growth at all in the 4 ppm. series, regardless of the amount of sulfur applied. The greatest weight was attained with a 10:1 ratio, but this was only 2 per cent of the control.

Although the application of sulfur reduced the toxicity of selenium, it is evident that complete antagonism was not approached. The leaves of the plants supplied with selenium were fewer and smaller than those of the controls. Applications of sulfur were beneficial but did not completely overcome the selenium injury. Some chlorotic leaves were found on all plants receiving selenium except those with the three highest sulfur:selenium ratios in the 1 ppm. series. The degree of chlorosis varied in each series.

Accumulation of selenium.—In addition to the beneficial effects of sulfur on growth, it has been shown that this element reduces the amount of selenium accumulated by plants (Hurd-Karrer and Kennedy, 1936). Growth of tobacco was depressed to such an

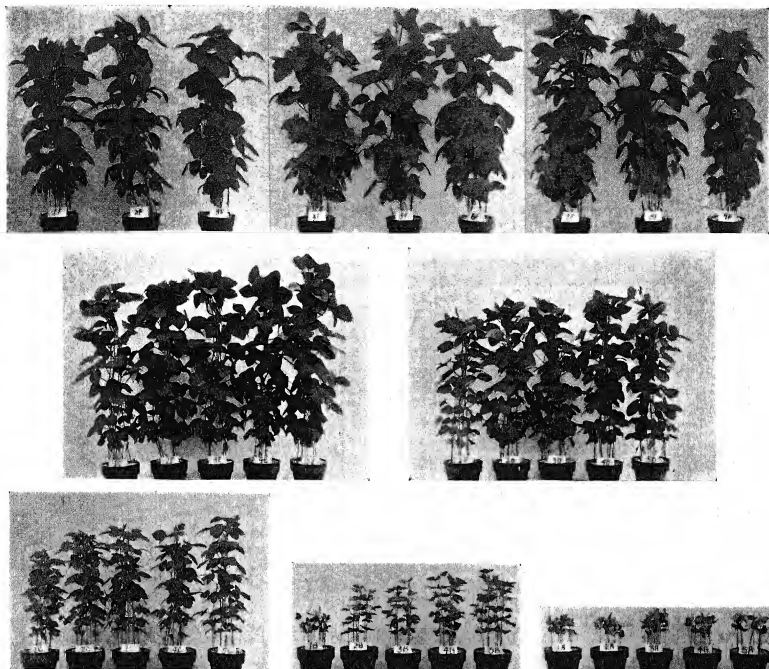


Fig. 4. The toxicity of selenium to soy bean plants grown in the greenhouse for 8 weeks. Series F (above) had no selenium, but the sulfur content varied from 0 to 160 ppm. Series E (center, left) received 0.5 ppm. of selenium; series D (center, right), 1 ppm.; series C (below, left), 2 ppm., series B (below, center), 4 ppm.; series A (below, right), 8 ppm. The culture at the extreme left in each series had no sulfur. The sulfur: selenium ratio in the other cultures increased from left to right 2.5:1, 5:1, 10:1, 20:1.

extent by the presence of selenium that the only series giving sufficient weights of plant material for analysis was the 1 ppm. series. A modification of Horn's codeine sulfate method (Martin, 1936) was used for determining the selenium content. Somewhat less poison (20–26 ppm.) was accumulated by plants which received sulfur than by those in the corresponding sulfur-free solutions (30 ppm.). The data are too limited, however, to support definite conclusions.

EXPERIMENTS WITH SOY BEANS.—Soy bean seeds of the Illini variety² were germinated in quartz sand in six-inch paraffined pots irrigated with distilled water. When all the pots had six seedlings about 5 cm. high, the additional seedlings were removed, and irrigation with the culture solutions was started. Concentra-

tions of selenium applied ranged from 0.5 to 8 ppm. The experiment was continued for 56 days; other conditions were similar to those in the tobacco experiment.

Injury and evidence of toxicity.—In general selenium in low concentrations was not so toxic to soy beans as to the other crop plants tested. Photographs taken prior to harvesting are shown in figure 4. Symptoms observed in severe cases included chlorosis, cupping and dwarfing of leaves, stunting of stem growth, retardation of blossom formation, and reduction of dry weight. Wart-like protuberances on the stems and deep lesions on the roots were also seen. The severity of these symptoms depended upon the concentration of selenium present in the solution.

Toxicity in the absence of sulfur.—The injurious effects of 0.5 to 1 ppm. of selenium, even without sulfur, were slight, and the plants remained normal

² The soy bean seeds were kindly supplied by Dr. W. L. Burlison of the University of Illinois.

in appearance during the entire growing period. The dry weights, however, of these cultures were reduced to 93 per cent and 69 per cent, respectively, of those for the selenium-free control cultures (fig. 2).

The application of 2 ppm. of selenium brought about a marked stunting of the plants, a slight reduction in leaf size, and retardation in blossom formation. Although the leaves were lighter in color than those of the control, they were not markedly chlorotic. The roots of these plants, when harvested, had numerous deep longitudinal lesions that had become covered with a rough, dark-brown tissue. The dry weight was only 22 per cent of that of the control culture.

Both 4 ppm. and 8 ppm. of selenium produced all the toxicity symptoms mentioned above. The dry weights for the two cultures were only 6 per cent and 4 per cent, respectively, of those for the controls. Leaves that had already developed turned from the normal green color to a yellowish green within a week after applying the culture solutions. In some cases leaves that developed after the solution was applied retained a normal green color for a few days before chlorosis became evident; in other cases the newly expanding leaves were chlorotic. The intervenous regions of the younger leaves were most severely affected; the veins always remained dark green. Growth at the margins of the yellowing leaves ceased, while the inner areas continued to grow; this resulted in a cupping of the leaves. Occasional pink spots appeared on the yellow regions. No blossoms formed in either of these cultures. Numerous small tubercles, about 0.5 mm. in diameter and evenly distributed over the stems, had appeared by the forty-fifth day. They developed only on severely poisoned plants, which, when analyzed, showed high concentrations of selenium. Tubercles have not previously been reported as characteristic of selenium injury of plants. The roots exhibited longitudinal lesions. Although the roots did not die, their injured condition was evident when they were compared with the smooth, light-brown roots of the control plants. This symptom in soy bean roots was different from the stunting of wheat and buckwheat roots previously reported.

Effect of the sulfur on toxicity.—It is evident from the curves of figure 2 that soy beans did not respond so favorably to the sulfur treatment as did the other kinds of plants studied. A solution containing 0.5 ppm. of selenium, without sulfur, gave a dry weight which was 93 per cent of the corresponding sulfur-free, selenium-free control. Adding sulfur to the solution increased the weight, but not the percentage yield in terms of the control. With 1 ppm. of selenium, also, the greatest dry weight on a percentage basis (69 per cent) occurred in the sulfur-free selenium culture. Root lesions were not present in the cultures that received sulfur.

In the series with 2 ppm. of selenium the dry weights of the plants were increased from 22 per cent to 35 per cent of the controls when the sulfur : selenium ratio was 5 : 1. The dry weights, as well

as the amounts of antagonism, were nearly the same with the three highest sulfur : selenium ratios. Applications of sulfur increased leaf size and reduced chlorosis and root injury.

When the solution contained 4 ppm. of selenium, the addition of sulfur brought about a gradual increase in the dry weights, from 6 to 14 per cent of the controls. Thus, greatest growth and antagonism were secured with the highest sulfur : selenium ratio (20 : 1). Chlorosis and root lesions persisted throughout all cultures in this series. Stem intumescences, though present in the cultures with the lower ratios, were absent in those with ratios of 10 : 1 and 20 : 1.

No apparent benefits were derived from adding sulfur to cultures receiving 8 ppm. of selenium. The dry weights, which were only 3-4 per cent of the controls, were not appreciably changed, and all the plants had lesions and stem intumescences.

Accumulation of selenium.—The soy bean plants grown in this experiment were ground into a fine powder and analyzed for selenium content by the codeine sulfate method. Figure 5 shows the effect of sulfate upon the amount of selenium accumulated by the plants. In general with each concentration of selenium used, the amount accumulated by the plant varied inversely with the concentration of sulfate in the culture solution. In the absence of sulfur, plants in 8 ppm. of selenium accumulated 180 ppm., but an increase in the sulfur : selenium ratio to 20 : 1 reduced the selenium content to 104 ppm. Similar, though in general less marked, reductions occurred with the other series of concentrations. With 4 ppm. of selenium in the culture solution the addition of sulfur reduced the selenium content of the plants from 72 ppm. to 50 ppm. With 2 ppm. there was a reduction from 63 ppm. to 49 ppm. In the series with 1 ppm. in the culture solution, the selenium content of the plants was irregular, owing perhaps to error in sampling. The effect of antagonism seems to have been most pronounced in the 0.5 ppm. series, in which the content was reduced from 55 ppm. to 17 ppm. when the highest concentration of sulfur was present.

These analyses of plants raised in solutions with various concentrations of selenium indicate that in general each successive increase in sulfur concentration tends to reduce the amount of selenium accumulated by the plants. The greatest reduction in selenium content, however, was to only about one-third the amount accumulated in the absence of sulfur. Although the sulfur-selenium antagonism thus tended to decrease the accumulation of selenium, the reduction in poison content of the plants was not sufficient to render them non-toxic to animals, even though the plants, while growing, showed no symptoms of injury (Martin, 1936; Schneider, 1936).

The results of these experiments illustrate the limitations which exist in the addition of sulfur as a means of preventing plants from absorbing toxic quantities of selenium. Under field conditions Franke and Painter (1937) observed that applications of sulfur and gypsum to a naturally seleniferous soil did not

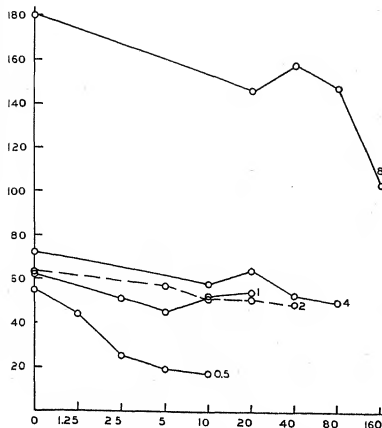


Fig. 5. Selenium accumulation by soy bean plants when raised in different concentrations of selenium with sulfur varying in each concentration. Abscissas represent ppm. of sulfur (as sulfate), ordinates represent ppm. of selenium accumulated. Numbers at the ends of the curves indicate the ppm. of selenium (as selenite) in the culture solutions.

inhibit the absorption of selenium by corn and wheat. In fact, the soils of the seleniferous areas are already saturated with sulfur in the form of gypsum, and it seems unlikely that the addition of more sulfur would be of any particular value in reducing the absorption

of selenium by the vegetation growing on these soils. Beath, Eppson, and Gilbert (1937) have shown that the application of sulfur in various forms may actually increase the absorption of selenium from a soil which derives its selenium from ground seleniferous plants.

SUMMARY

Tobacco was more severely injured than soy beans by selenium (as sodium selenite) supplied by means of a continuously renewed nutrient solution to plants growing in sand cultures. Stunting was the only symptom of selenium injury uniformly developed by tobacco. Soy bean plants exhibited root lesions and stem intumescences, not previously described as symptoms of selenium injury, in addition to the usual stunting and chlorosis found in other plants.

The presence of sulfur (as sulfate) in the lower concentrations of selenite tended to decrease the toxic effects of the latter, but in no case was antagonism sufficient to bring about growth equaling that of the controls.

The amount of selenium absorbed by the plants was directly related to the selenite concentration of the nutrient solution. The addition of sulfate to the nutrient solution tended to reduce the accumulation of the selenium in the plant. The greatest decrease in selenium content, however, was to one-half or one-third the amount present in the absence of sulfate. With the concentrations of selenite used, this reduction was not sufficient to render the plants incapable of possible injury to animals.

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THE SOMATIC CHROMOSOME COMPLEMENT OF *HABRANTHUS ROBUSTUS*¹

Walter S. Flory

MEMBERS of the Zephyrantheae have received but slight cytological attention. The small number and favorable size of the chromosomes of *Habranthus robustus* Herbert, of this tribe, were noted in the course of some routine chromosome counts. Since the cytological evidence also lends support to recent taxonomic contentions, it seems desirable to report the facts at hand.

The chromosome descriptions reported here deal with external morphology and are based on studies of root-tip cells in smear preparations. The preparations were made after Warmke's (1935) method and Brown's (1937) modification of it. The chromatin so nearly fills the cell in division that it is necessary to flatten the cells, often to the bursting point, in order

may be grouped into 3 types of two members each—i.e., I and II, III and IV, and V and VI. The members of any of these three types may be easily selected in any clear plate. With a little study, the two members of each type may be distinguished. Some of the more apparent differences are those that exist in total length and in the positions of the spindle-fiber attachment constrictions. I and II are the longest chromosomes and have an approximately median constriction point; III and IV are next in length and have sub-median constrictions; the shortest chromosomes, V and VI, have the most nearly terminal attachment constrictions. The general relationship that is apparent between length and the constriction position in going from longest to shortest

TABLE 1. *Characteristics of the chromosomes of Habranthus robustus.*
(Figures averaged from measurements on eight metaphase plates.)

Chromosome	Length in microns			Ratio of arms (approximate)	Constriction position approximates	Other characters often aiding identification
	Long arm	Short arm	Total			
I	12	10	22	1:1	median	U- or V-shaped; ends curving outward
II	10	9	19	1:1	median	U- or V-shaped; ends curving inward
III	10	7.5	17.5	1.5:1	submedian	U-shaped; or zigzagged into a double, reverse U
IV	10	5	15	2:1	submedian	Long constriction, usually $0.5 + \mu$; short arm irregularly zigzagged with end pointing in J-shaped; usually an acute angle between arms
V	11	3.5	14.5	3:1	subterminal	J-shaped; differs from V in arms forming less acute angle
VI	10	4	14	2.5:1	subterminal	

to have the chromosomes separated enough for individual study. It was found that when 5 or 10 per cent of concentrated HCl was added to the alcohol-acetic acid fixative, the cells separated more easily, and flattening of them was facilitated. The fixative containing the HCl caused a measurable shrinkage of the chromatin as compared with the usual fixative.

The six pairs of chromosomes have distinctions by which they may be recognized at prophase, metaphase (fig. 1, 2, 4), and anaphase (fig. 3, 5). Table 1 gives the characteristics of the six chromosomes which have here been arranged according to total length. The figures on length have been averaged from measurements on eight metaphase plates. The chromosomes

chromosomes—i.e., that the constrictions become increasingly distant from the median—is of decided help in distinguishing the members of the three different groups. This tendency can be seen by comparing the data in the fourth and fifth columns (from the left) in table 1. The fourth column gives the average total length of the different chromosomes, while the adjoining figures in the fifth column approximate the average length ratio between the long and short arms of the respective chromosomes. The right-hand column of table 1 gives other characteristics which aid in picking out the several members of the complement. These may not prove as constant as the factors of length and point of attachment constriction, but knowledge of them is of distinct advantage in questionable cases. There are secondary constrictions which may prove to be definite in location on certain chromosomes and which would then furnish

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further identifying marks. No satellited chromosomes have been observed in either metaphase or anaphase figures. However, occasionally in prophase a chromosome bearing a satellite is seen. Such a chromosome is shown at the point of the arrow in figure 6. To the lower left, with its end on the nucleolus, which is partly out of focus, is a chromosome of the same length and



Fig. 1-3. Somatic chromosomes of *Habranthus robustus*. From camera-lucida drawings. Original magnification $\times 3400$, reduced to $\times 1005$ in reproduction.—Fig. 1. Metaphase plate showing six pairs of chromosomes. Fixative contained 10 per cent HCl.—Fig. 2. Metaphase plate. No HCl in fixative.—Fig. 3. Anaphase, showing 12 chromosomes going toward each pole of the spindle. Fixative contained 10 per cent HCl.

with the same arm-length ratio. This can not be seen to possess a satellite but is probably the homologue of the satellited chromosome. The chromosome with the satellite is apparently III.

In figure 7 the six chromosomes of the complement are arranged linearly. Those in the top row are camera-lucida drawings from a metaphase plate; the bottom line is from an anaphase stage. These are characteristic and depict rather clearly the descriptive points discussed above and summarized in table 1.

DISCUSSION.—The six chromosome pairs range in length from 14 to 22 microns (table 1) with an average of about 17 microns. In size these compare favorably with other large-chromosomed plants. (According to Sax and Sax, 1935, the average length in microns of somatic chromosomes at metaphase is: in *Vicia faba*, 13 μ ; in *Lilium regale*, 22 μ ; in *Tradescantia* sp., 21 μ ; in *Secale cereale*, 14 μ ; in *Trillium grandiflorum*, 40 μ .) In prophase and at early metaphase the coiling of the chromatids can be rather clearly followed. The chromosome complement of this plant, then, would seem to furnish promising material for an analysis of chromosome structure.

In addition to pointing out another species with chromosomes of a size and character favorable for analytical study, this work presents evidence which has some bearing on taxonomic questions. The species and genus concerned have had a tumultuous nomenclatorial history during the more than a hundred years of their existence. The following brief outline of this history has been made possible by information which Dr. H. Harold Hume has generously given from his extensive notes on the genera involved. The combination *Habranthus robustus* was first made in 1829 by William Herbert, who had established the genus five years earlier. This species was placed, with all Herbert's other *Habranthus* species, in *Hippeastrum* by Baker (1878), at which time it became *H. tubispathum*. In his monograph of 1888, Baker concurred with Bentham and Hooker (1883) in their division of Herbert's *Habranthus* between the genera *Hippeastrum* and *Zephyranthes*. At this time the species with which we are here concerned became *Z.*

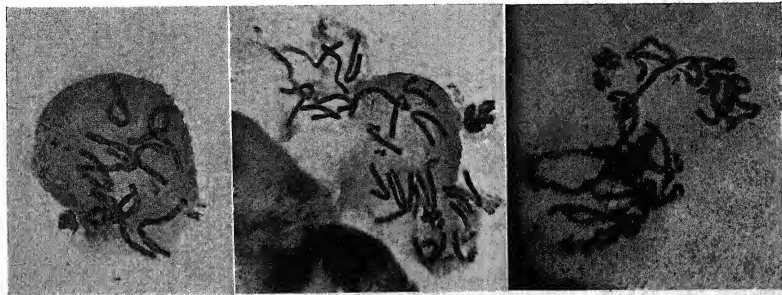


Fig. 4-6. Microphotographs of chromosomes from the root tip of *Habranthus robustus*. Original magnification $\times 1000$.—Fig. 4 (left). Photograph of the metaphase plate drawn in fig. 1.—Fig. 5 (center). Photograph of the figure drawn in fig. 3.—Fig. 6 (right). Photograph of a prophase stage showing a satellited chromosome at the point of the arrow. Note how the chromatin almost fills the cell and how the cells burst if the chromosomes are spread out.

robusta. The original combination *Habranthus robustus* Herb. was restored when Stapf (1927) recognized Herbert's concept of these genera as the most natural and rational one. The authenticity of this conception was recently confirmed by Sealy (1937) whose studies convince him that *Habranthus* is deserving of independent generic status. The work of

Stapf and of Sealy came to the writer's attention after his original report on the chromosomes of this species (Flory, 1937).

But few chromosome numbers have been reported in the tribe Zephyrantheae of the Amaryllidaceae. In addition to one species of *Sternbergia* with which we are not interested here, Yamamoto and Hosono (1931) list *Zephyranthes candida* as having $2n=36$; Nagao and Takusagawa (1932) give $n=19$, $2n=38$ for this form, and $n=46$ in *Z. carinata*; and *Z. Lindleyana* has $2n=48$ according to Fernandes (1930). In 1913 Pace gave $n=12$ and $2n=24$ for *Z. texana*. The first three species are true *Zephyranthes*, but if *Habranthus* be reinstated as a genus *Zephyranthes texana* becomes *Habranthus texanus*. In *H. robustus* $2n=12$. Cytologically this would indicate a close relationship between this form and *H. texanus* and a more distinct affinity to the three species of *Zephyranthes*. Thus, even though fragmentary and somewhat limited, the cytological facts support recent taxonomists in their conclusions that *Habranthus* should be separated from *Zephyranthes* and given generic rank. At the same time a field for future cytotaxonomical study is suggested.

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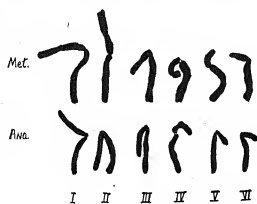


FIG. 7. Linear arrangement of the six chromosomes of *Habranthus robustus*. Camera-lucida drawing made at an original magnification of $\times 3400$. Reduced to $\times 1005$ in reproduction. Metaphase chromosomes above, anaphase below. From left to right the chromosomes decrease in size, while, in general, their spindle fiber connections become increasingly remote from a median position.

INFLUENCE OF INDOLE-3-ACETIC ACID ON THE RESPIRATION AND GROWTH OF INTACT WHEAT SEEDLINGS¹

Robertson Pratt

INVESTIGATION of the influence of auxins on different physiological processes constitutes an important, but as yet relatively unexplored, aspect of plant hormone research. Although the ultimate effect of auxins is usually measured in terms of cell or organ enlargement, little is known regarding the mechanism by which the final result is attained.

Accelerated respiration might be expected to accompany increased growth, especially if this involves cell multiplication and increase in protoplasm in addition to mere enlargement of cells. Several investigators, however, have failed to detect any accelerating influence of auxin on respiration; they noted retardation when high concentrations were employed (Boysen-Jensen and Nielsen, 1925; Bonner, 1936;² Kögl, Haagen-Smit, and van Hulsen, 1936). Calculations led Thimann and Bonner (1933) to conclude that auxins do not enter directly into the growth reactions of the *Avena* coleoptile but "act in some indirect manner that allows each molecule to exert its influence a number of times."

The coleoptile of grass seedlings is an ephemeral structure with relatively low metabolic activity. In an attempt to discover the influence of auxins on metabolic processes of plants, it seems of interest, therefore, to investigate organs that are physiologically more active. The present experiments were performed in order to study the effect of indole-3-acetic acid, or heteroauxin, on the respiration and early growth of intact wheat seedlings. The work was done in the laboratory of plant physiology of Columbia University. The friendly and helpful criticism of Prof. Sam F. Trelease is appreciatively acknowledged.

MATERIALS AND METHODS.—Fresh solutions of crystalline indole-3-acetic acid (Eastman Kodak Co.) were prepared for each experiment by dissolving a suitable quantity in 1–2 cc. of 95 per cent ethyl alcohol and then diluting with water distilled from Pyrex flasks through quartz condensers.³ The stock solution was heated to evaporate the alcohol. Similar quantities of alcohol were added to the water to be used in control tests and were evaporated in the same way.

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² Bonner (1933) reported increased respiration of *Avena* coleoptile segments resulting from the addition of an auxin extract obtained from *Rhizopus strombosus* but later (1936) concluded that the stimulation previously observed was due to an impurity present in the extract.

³ No attempt was made to recrystallize or otherwise purify the heteroauxin. Since this substance is widely used by experimenters, it seemed desirable to study the physiological properties of the form sold by chemical supply houses and employed by most investigators.

Marquis wheat seeds (*Triticum vulgare*) of nearly uniform size were used in these experiments. Several sets of 20 seeds each were soaked 18–19 hours in 90 cc. of the respective solutions. At the end of that time the rate of O₂ consumption was measured by means of Warburg manometers and found to be approximately the same in all preparations (0.04 c.m.m. O₂/seed/min.). The temperature throughout the experiments—i.e., during soaking, respiration measurements, and incubation periods—was 29°C. Soaked seeds and seedlings were exposed to light only during the brief intervals required for transferring them to and from the respiration vessels. After the initial measurements of respiration, the seeds were placed on moist filter paper in Petri dishes and incubated for 44–46 hours. The respiration of 10 typical seedlings was then measured and found to be markedly influenced by the treatment to which the seeds had been subjected. Since preliminary experiments had shown that at this stage of development the embryos accounted for 98–99 per cent of the O₂ consumption of the seedlings, the embryos (chiefly roots and coleoptiles) were separated from the endosperm and dried for 18 hours at 63°C., and the respiration rates were calculated in terms of c.m.m. O₂ consumed per minute per mg. of dry weight.

RESULTS AND DISCUSSION.—Data compiled from the experiments are presented in tables 1–3 and figure 1.

The principal results of these tests are shown clearly by the two curves in figure 1. Over a relatively wide range of concentrations, growth decreased and the relative respiration rate increased approximately as linear functions of the logarithm of the heteroauxin concentration. From the lower, descending curve it may be seen that no concentration of heteroauxin employed improved the early growth of the seedlings, as measured by dry weight of the developing embryos. Smaller size was responsible for the decreased dry weight of the auxin-treated embryos. Wheat is probably an auxin-satiated plant (Greenfield, 1937; Marmer, 1937); so that upon addition of auxin its growth is inhibited by the toxic action of the chemical. But the rise in the upper curve shows that the intensity of respiration, per mg. of embryo weight, was greatly increased as the hormone concentration was raised from 0.01 ppm. (5.7×10^{-6} M) to 50 ppm. (2.85×10^{-4} M). The final rapid fall of the curve for respiration indicates the effect of treatment with solutions stronger than 50 ppm.; concentrations above 100 ppm. (5.7×10^{-4} M) caused a marked depression of respiration. Complete data for this experiment are given in table 1.

TABLE 1. *Respiration and growth of wheat seedlings previously soaked 18 hours in unbuffered solutions containing different concentrations of indole-3-acetic acid in distilled water and then incubated for 45 hours on moist filter paper.^a Temperature = 20°C.*

Concentration of heteroauxin			Respiration		Growth	
ppm.	M	pH	c.mm.O ₂ /mg./min.	Percentage of control in H ₂ O	Av. dry wt. of embryo, mg.	Percentage of control in H ₂ O
Control containing dist. H ₂ O only		6.2	0.215	100	3.77	100
0.01	5.7×10^{-8}	6.2	0.212	99	3.59	95
0.10	5.7×10^{-7}	6.1	0.259	121	3.01	80
1.00	5.7×10^{-6}	5.7	0.311	145	2.34	62
10.00	5.7×10^{-5}	4.7	0.345	161	1.72	46
50.00	2.85×10^{-4}	4.2	0.382	178	0.94	25
100.00	5.7×10^{-4}	4.0	0.273	127	0.92	24
150.00	8.57×10^{-4}	4.1	0.085	40	0.95	25
200.00	1.14×10^{-3}	3.9	0.089	41	0.88	23

^a After 18 hours of soaking the rate of respiration was 0.04 c.mm.O₂/seed/min. in all preparations.

It has already been mentioned that the influence of heteroauxin was not apparent until active growth began under aerobic conditions. No differences in respiration could be detected immediately after the soaking period. At that time the embryos were swollen, but rapid cell division and enlargement had not yet begun.

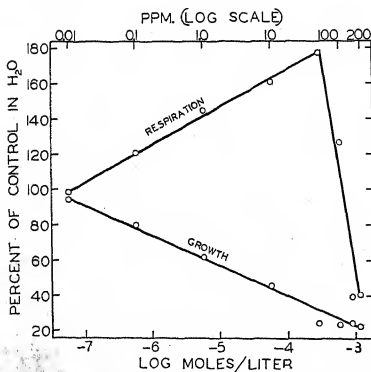


Fig. 1. Relative rates of respiration and growth of wheat seedlings after exposure of the seeds to different concentrations of indole-3-acetic acid in distilled water.

Although measurements made by means of a Beckman glass electrode disclosed a considerable range in the pH values of the different heteroauxin solutions (column 3, table 1), the tests recorded in table 2 demonstrated that the effects on growth and respiration shown in table 1 and figure 1 were not the direct result of changes in the H-ion concentrations of the heteroauxin solutions. Table 2 shows no pronounced difference in respiration rate or consistent variation

in the amount of growth when the pH values of buffered solutions lacking heteroauxin were raised from 3.3 to 6.8. Respiration was somewhat greater in the buffer solutions than in distilled water. The maximum increase, however, was only about one-ninth that brought about by the addition of 44 ppm. of heteroauxin.⁴

Since indole-3-acetic acid is weakly ionized, the fraction of the molecules in the undissociated state should increase as the pH value of the solution decreases (cf. Albaum and Kaiser, 1937). Therefore, if the compound penetrates living cells chiefly in the undissociated state, as evidence presented by Albaum, Kaiser, and Nestler (1937) seems to indicate, a given concentration of heteroauxin should exert a greater physiological effect at lower pH values than at higher ones. Marmer (1937) has already shown that a greater concentration of this substance is required in the external solution to produce a given amount of inhibition in the growth of wheat seedlings at pH 7.5 than at pH 4.6.

Several experiments were performed in an attempt to discover whether any direct relation could be detected between respiration and growth on the one hand and the concentration of undissociated heteroauxin in the external solution on the other.⁵ Uniform concentrations of heteroauxin were employed, and different pH values were obtained by the use of suitable buffer mixtures. Considerable quantitative variation was found among the different experiments, however, and it is believed that this phase of the work requires further study.

Maximum stimulation of respiration occurred after seeds were exposed to a solution containing 50 ppm.

⁴ The close agreement of the respiration values in this experiment was fortuitous, since the range of variation among several series of control plants was about 6 per cent—i.e., ± 3 per cent.

⁵ Calculations of the concentrations of undissociated heteroauxin are omitted from table 1, since they are considered to be unreliable for unbuffered solutions at the dilutions used.

($2.85 \times 10^{-4}M$) of heteroauxin, or indole-3-acetic acid, at pH 4.2. A solution of this strength contains 34.3 mg. of carbon per liter. The data in table 3 indicate, however, that the increased rate of respiration after treatment with heteroauxin solutions was not due directly to the additional carbon supplied to the seed-

ured by means of Warburg manometers. The dry weights of the embryos (chiefly roots and coleoptiles) were determined, and the respiration rates were expressed in terms of c.mm. O_2 consumed per mg. of dry weight of embryo (the latter accounting for about 98 per cent of the total respiration of the seedling).

TABLE 2. *Respiration and growth of wheat seedlings previously soaked 19 hours in solutions buffered at different pH values^a and then incubated for 45 hours on moist filter paper.^b Temperature = 29°C.*

pH	Respiration		Growth	
	c.mm. O_2 /mg./min.	Percentage of control in H_2O	Av. dry wt. of embryo, mg.	Percentage of control in H_2O
3.3	0.213	108	2.59	72
4.4	0.206	104	3.46	97
5.3	0.216	109	3.09	86
6.8	0.205	103	3.36	94
Dist. H_2O pH = 6.2	0.198	100	3.58	100
44 ppm. heteroauxin (unbuffered: pH = 4.3)	0.359	182	1.10	31

^a Buffers were prepared by mixing solutions of 0.025 M citric acid and 0.05 M K_2HPO_4 in suitable proportions.

^b After 19 hours of soaking the rate of respiration was 0.04 c.mm. O_2 /seed/min. in all preparations.

lings. Representatives of different classes of substances (hexose, amino acid, potassium salt of organic acid, and simple organic acid) were employed in addition to indole-3-acetic acid in preparing solutions containing 34.3 mg. of carbon per liter. None of these substances brought about a change in respiration or growth comparable to that produced by the heteroauxin. It may be noted, however, that the acetate-containing compounds accelerated respiration and depressed growth more than the other substances tested. Further work would be needed to determine whether this result has special significance.

SUMMARY

Wheat seeds were soaked 18-19 hours in buffered and unbuffered solutions of heteroauxin (indole-3-acetic acid) and were allowed to germinate on moist filter paper in Petri dishes for 44-46 hours. The oxygen consumption of the seedlings was then meas-

Seedlings were exposed to light only during the brief intervals required to transfer them to and from the respiration vessels. The temperature in all experiments was 29°C.

No differences in respiration were detected immediately after the period of soaking, but marked differences were found after rapid growth began.

Concentrations from 0.1 ppm. ($5.7 \times 10^{-7}M$) to 100 ppm. ($5.7 \times 10^{-4}M$) of heteroauxin markedly accelerated respiration per unit dry weight of embryo and strongly depressed growth. The maximum increase in respiration followed soaking the seeds in a solution containing 50 ppm. ($2.85 \times 10^{-4}M$) at pH 4.2. The rate was nearly double that for the untreated controls.

The acceleration of respiration was not the direct result of changes in the pH of the external solution, but was due to the heteroauxin.

The higher oxygen consumption was not due directly to the additional carbon furnished by the heteroauxin,

TABLE 3. *Respiration and growth of wheat seedlings previously soaked 19 hours in solutions containing 34.3 mg. carbon per liter and then incubated for 46 hours on moist filter paper.^a Temperature = 29°C.*

Substance	Molar concentration	pH	Respiration		Growth	
			c.mm. O_2 /mg./min.	Percentage of control in H_2O	Av. dry wt. of embryo, mg.	Percentage of control in H_2O
Indole-3-acetic acid .	2.85×10^{-4}	4.2	0.375	189	1.70	46
Glucose	4.76×10^{-4}	6.0	0.183	92	3.60	97
Glycine	14.28×10^{-4}	6.0	0.176	89	3.60	97
K-acetate	14.31×10^{-4}	6.5	0.229	116	2.80	76
Acetic acid	14.28×10^{-4}	3.9	0.268	136	2.40	65
Control containing dist. H_2O only		6.2	0.198	100	3.70	100

^a After 19 hours of soaking the rate of respiration was 0.03-0.04 c.mm. O_2 /seed/min. in all preparations.

since other types of carbon compounds had little effect on respiration.

These results indicate that heteroauxin is capable of markedly accelerating the metabolism of plant organs of high physiological activity.

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TWO NEW SAPROLEGNIACEOUS FUNGI¹

Alfred F. Bartsch and Fred T. Wolf

WHILE COLLECTING aquatic fungi and algae in the vicinity of Madison, Wisconsin, two unusual Oomycetes belonging to the Saprolegniaceae were found parasitizing aquatic animals. One, a species of the genus *Aphanomyces*, occurs as a parasite of the protozoan *Acineta flava*; the other, presumably a species of *Hydutinophagus*, was found within the bodies of a rotifer, *Monostyla* sp. indet. Inasmuch as both these unique fungi appear to be undescribed, it became of interest to make a study of their development and life history.

The suetorian *Acineta flava* was found growing upon filaments of *Cladophora glomerata* in the Yahara River on February 19, 1937. When this material was brought into the laboratory, it was found that the *Acineta* was attacked by a species of *Aphanomyces*.

Infection of *Acineta flava* occurs by means of zoospores of the fungus, which encyst upon the basal portion of the body of the host and give rise to germ tubes which penetrate the lorica (fig. 1). As many as fourteen germ tubes have been seen within a single individual; multiple infections are apparently com-

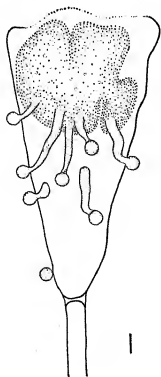
mon. The germ tubes develop within the protoplast of the host to form an extensive, branched intramatrix mycelium composed of non-septate hyphae 3.7-9 μ in diameter (fig. 2). The protoplasmic contents of the protozoan are gradually utilized in the nourishment of the fungus.

Asexual reproduction of the fungus occurs by means of sporangia which protrude from the naked anterior portion of the host or occasionally push through the lorica (fig. 3). The sporangia are relatively undifferentiated from the mycelium, 5-6 μ in diameter, up to 70 μ in length, and contain a single row of spores. Upon discharge, the 9-16 zoospores become encysted in an irregular clump at the mouth of the sporangium; the encysted spores are 4-7 μ in diameter (fig. 5). After several hours, the cysts germinate to release motile biciliate zoospores (fig. 7). The empty cysts may remain clustered at the mouth of the sporangium (fig. 6). After swimming about actively for a time, the zoospores again encyst and may cause infection of other individuals of *Acineta*. Later, sexual organs of the fungus are formed within the body of the protozoan (fig. 4). The oogonia are spherical or slightly irregular in form and have a smooth wall (figs. 8, 9). They range from 18 to 24 μ in diameter, averaging 21 μ . Each oogonium contains a single oospore 15-22 μ (average 18 μ) in diameter. The antheridia are declinous in origin. Usually one or

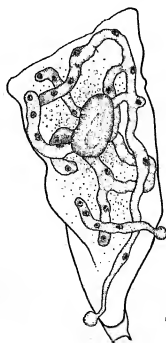
¹ Received for publication February 19, 1938.

This investigation was carried out under the direction of Dr. E. M. Gilbert, during the tenure of Alumni Research Fellowships by the writers. We are very grateful to Dr. F. K. Sparrow, Jr., of the University of Michigan, for the loan of Valkunov's Bulgarian paper.

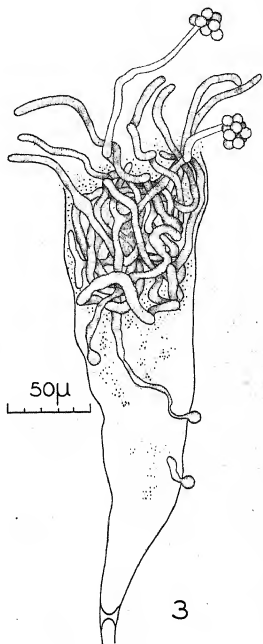
Fig. 1-11. All drawings were made with the aid of an Abbé camera lucida, using a 10 \times ocular and 4 mm. and 1.8 mm. objectives. Original magnification of figures 1-4, 700 \times ; (absolute scale near fig. 3); figures 5-11, 1600 \times ; (absolute scale between fig. 9 and 11): reduced about one-half in reproduction.—Fig. 1-9. *Aphanomyces acinetae* in *Acineta flava*.—Fig. 1. Protozoan showing method of infection.—Fig. 2. Protozoan showing intramatrix mycelium of the fungus.—Fig. 3. Protozoan showing sporangia of the fungus.—Fig. 4. Protozoan showing oogonia and antheridia of the fungus.—Fig. 5. Sporangium shortly after zoospore discharge, showing encysted zoospores at the tip of the sporangium.—Fig. 6. Sporangium at a later stage, showing the empty cysts.—Fig. 7. Zoospore.—Fig. 8. Oogonium and antheridium, showing the fertilization tube.—Fig. 9. Sexual organs within the host.—Fig. 10-11. *Hydutinophagus americanus* in *Monostyla* sp.—Fig. 10. Rotifer showing the intramatrix mycelium of the fungus.—Fig. 11. Rotifer showing oogonia of the fungus.



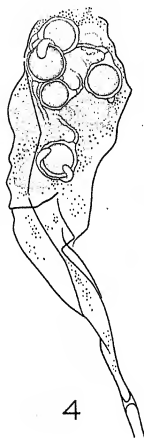
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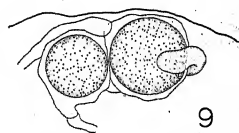
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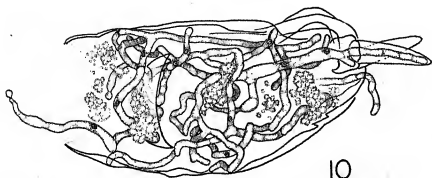
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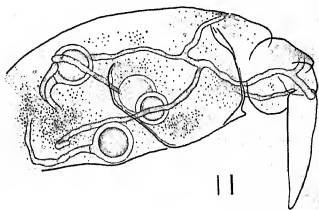
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occasionally two or three occur on every oogonium; the fertilization tube has been seen in a few instances.

These observations indicate that the fungus is a member of the genus *Aphanomyces* deBary (1860). Inasmuch as it appears not to have been described previously, the name *A. acinetophagus* is proposed for it:

Aphanomyces acinetophagus sp. nov.—Plantis in protozoa (*Acineta flava*) parasitice, hyphis inficentibus numerosis acque in lorica in eorum corporibus penetrantibus. Mycelio intramatro, copioso, ramoso, 3.5–9 μ diam. Sporangis ex hyphis non distinctis, 5–6 μ diam. usque 70 μ longis, e parte anteriore nuda corporis vel interdum e lorica extantibus. Zoosporis monoseriatis continentibus, primatus ab apicibus sporangiorum irregulariter aggregatis; sporis immotis 4–7 μ , plerumque 6 μ diam.; dein mobilibus et cilia bina gerentibus, et denique hyphas emittentibus. Oogonis in corporibus *Acinetae* sphaericis vel paulo irregularibus; 18–24 μ , plerumque 21 μ diam., membrana oogoniorum levi, oospora unica, 15–22 μ , plerumque 18 μ diam. Antheridia in eodem oogonio declinatis, 1–2–3 pro quoque oogonio efformatis, papillis evacuationis munitis. Hab. in *Acineta flava*. Yahara River, Madison, Wis.

Parasite within the protozoan, *Acineta flava*. Infection resulting from the germination of spores on the lorica of the basal portion of the host; multiple infections common. Intramatrix mycelium extensive, branched, the hyphae 3.7–9 μ in diameter. Sporangia undifferentiated, 5–6 μ in diameter, up to 70 μ in length, protruding from the anterior naked portion or occasionally through the lorica of the host. Spores arranged within the sporangium in a single row; upon discharge encysting at the mouth of the sporangium in an irregular clump; encysted spores 4–7 μ , averaging 6 μ in diameter; later escaping from the cysts as motile biciliate zoospores, which again encyst and germinate by germ tube. Oogonia formed within the host, spherical or slightly irregular, 18–24 μ , averaging 21 μ in diameter, with a smooth wall and containing a single zoospore 15–22 μ , averaging 18 μ in diameter. Antheridia declinuous, usually one, occasionally two or three, present on every oogonium; fertilization tube visible. Type locality: Yahara River, Madison, Wis.

A collection of water from Lake Wingra on October 18, 1937, yielded a sterile species of *Achlya*. This culture was neglected for a time, and the *Achlya* hyphae were attacked by numerous chytrids. Furthermore, within some of the hyphae were found a number of rotifers belonging to the genus *Monostyla*. The bodies of the rotifers were filled with the mycelium of a saprolegniaceous fungus. Inasmuch as the fungus was not observed to attack living rotifers, it was impossible to determine whether or not it is actually parasitic.

Infection of *Monostyla* presumably occurs following the germination of spores of the fungus within the stomach of the rotifer. The germinating spores give rise to an extensive, profusely branched mycelium which entirely fills the body of the rotifer (fig. 10). The mycelium of the fungus is composed of small non-septate hyphae 2–4 μ in diameter.

Unfortunately, the sporangia and spores of the *Monostyla* fungus were not observed in the limited amount of material available. Spherical oogonia 8.5–11.2 μ in diameter are formed within the host (fig. 11). Each oogonium contains a single oospore 7–9 μ in diameter. Antheridia are apparently absent.

Although the asexual reproductive structures were not observed, it seems probable that this organism should be placed in the genus *Hydatinophagus* Valcanov (1931b), based on the single species *H. apsteinii* which parasitizes the rotifer *Hydatina*. The Wisconsin fungus differs from *H. apsteinii* in two important features. It has no antheridia, which are present in the type species, and attacks a different host, *Monostyla*. The fungus attacking *Monostyla* is therefore described under the name *H. americanus* sp. nov.

Hydatinophagus americanus sp. nov.—Plantis in corpore *Monostylae* sp. (Rotatoria). Adparenter germinationis sporarum in corpore orundis, et deinde hyphis inficentibus corpores penetrantibus. Mycelio intramatro, diffuso, copiose ramoso, corporibus animalculae (*Monostyla*) cum hyphis repletis; hyphis 2–4 μ diam. Sporangis et sporis non observanda. Oogonis in corporibus animalcularum, sphaericis, 8.5–11.2 μ diam.; oospora singula, 7–9 μ diam., antheridis ignotis. Hab. in *Monostyla* sp. (Rotatoria), Lake Wingra, Madison, Wis.

Occurring within the rotifer *Monostyla* sp. Infection apparently following germination of spores within the stomach of the rotifer. Intramatrix mycelium extensive, profusely branched, entirely filling the body of the rotifer; hyphae 2–4 μ in diameter. Sporangia and spores not observed. Oogonia formed within the host, spherical, 8.5–11.2 μ in diameter, containing a single oospore 7–9 μ in diameter. Antheridia not observed. Type locality: Lake Wingra, Madison, Wis.

DISCUSSION.—Although most of the fungi belonging to the Saprolegniaceae are saprophytes, the genus *Aphanomyces* shows a decided tendency toward a parasitic mode of existence. Various species of *Aphanomyces* are parasitic upon numerous higher plants, including peas, beets, radishes, oats, and tomatoes, upon other Phycomycetes such as *Achlya* and *Pythium*, and on the conjugate algae *Spirogyra* and *Zygnema*. In addition, a few members of the genus have been reported as parasitizing animals. *Aphanomyces Magnusii* (Schikora, 1922) and *A. astaci* (Rennerfelt, 1936) cause a destructive disease of crayfish in Europe known as the "Krebspest." Gicklhorn (1923) has described a parasite of the eggs of the copepod *Diaptomus* under the name *A. ovidestruens*. *Aphanomyces acinetophagus*, however, is the only member of the family which has been described upon a protozoan host.

Inasmuch as a number of Oomycetes are known to attack rotifers, their taxonomy presents a more difficult problem. *Synchaetophagus* Apstein (1910), based on a single species, *S. balticus*, parasitizing the marine rotifer *Synchaeta monopus*, is of somewhat uncertain systematic position because the zoospore behavior and type of sexual reproduction were not observed.

It is placed in the Saprolegniaceae by Valkanov (1931b, 1932).

The genus *Zoopagus* Sommerstorf (1911) has been studied by Sparrow (1929) and placed by him in the Pythiaceae. *Sommerstorfia* Arnaudow (1923), like *Zoopagus* has specialized hyphae with mucilaginous tips, which adhere to the mouth parts of the rotifer. The Wisconsin fungus is obviously not cogenetic with any of these.

Valkanov (1931a) described a parasite of *Hydatina senta* in Bulgaria under the name *Aphanomyces hydatinae*. In a later paper (1931b) this fungus was made the type of a new genus, *Hydatinophagus*. The Bulgarian diagnosis (which is practically identical with the German summary, although it is not clear that the latter is to be considered as a formal diagnosis) contains only one distinctive character. *Hydatinophagus* is described as beginning its development in the stomach of the rotifer, presumably following ingestion of spores, rather than by capturing the rotifer by specialized hyphae as in *Zoopagus* and *Sommerstorfia* or by developing from a germinating spore on the surface of the animal as in *Synchaetophagus*.

It would appear that *Hydatinophagus* is to be separated from *Aphanomyces* only with difficulty. Valkanov (1932) found that the oogonia of *Hydatinophagus* contains a central vacuole from the beginning, while Kasanowsky (1911) observed that in the oogonia of *Aphanomyces laevis* the vacuole develops later, the oogonium originally being filled with dense cytoplasm. This difference does not appear to be an

important one. Otherwise, the cytological features of the development of the sporangia and sexual organs of *Hydatinophagus* are similar to those in *Aphanomyces*.

It is primarily because of the method by which the host becomes infected that we are referring the fungus attacking *Monostyla* to the genus *Hydatinophagus*.

Although Valkanov made a careful cytological study of *H. apsteinii*, in none of his papers are measurements of the hyphae, sporangia, or sexual organs to be found. In view of the fact that this information is lacking, that *H. apsteinii* has been reported only from Bulgaria, that the Wisconsin fungus lacks antheridia, which are present in *H. apsteinii*, and that the rotifers attacked by the two fungi belong to different genera, it has seemed desirable to create a new species, *H. americanus*, for the fungus attacking *Monostyla* in Wisconsin.

SUMMARY

Two hitherto undescribed Oomycetes have been found in the vicinity of Madison, Wisconsin. The life history of a parasite of the protozoan *Acineta flava* is given, and the fungus is described as *Aphanomyces acinetophagus* sp. nov. A fungus attacking the rotifer *Monostyla* sp. is apparently related to the genus *Hydatinophagus* and is described as *H. americanus* sp. nov.

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HYBRIDIZATION IN TRADESCANTIA. III. THE EVIDENCE FOR INTROGRESSIVE HYBRIDIZATION¹

Edgar Anderson and Leslie Hubricht

PREVIOUS STUDIES of the American species of *Tradescantia* have shown that interspecific hybridization is comparatively frequent between the eighteen or more species closely related to *Tradescantia virginiana* (Anderson and Sax, 1936; Anderson and Woodson, 1935). Hybridizations which were inferred from herbarium and field work have been produced experimentally in the breeding plot (Anderson, 1936a). Detailed morphological analyses of hybrid populations have shown that the ultimate effects of interspecific hybridization are various (Anderson, 1936c). Apparently the commonest result is that through repeated back-crossing of the hybrids to the parental species there is an infiltration of the germplasm of one species into that of another. If, for instance, two species, 'A' and 'B' come into effective contact, they usually do so under conditions which greatly favor either 'A' or 'B.' If 'A' and 'B' differ in habitat preferences, seldom or never is there a habitat equally acceptable to both; they usually meet, if at all, in a situation quite favorable to one of the species but just fairly so to the other. Therefore, if hybrids are produced, they tend to cross back to the more abundant species. The progeny of these secondary hybrids are likewise crossed back again, and so on. The final result will depend upon the balance between the deleterious effects of the foreign germplasm and its advantageous effects in the areas where the hybridization has taken place or to which the hybrids may spread.

Preliminary analyses of a number of genera of the flowering plants (Anderson, 1936b; Riley, 1936, 1937; Goodwin, 1937; Delisle, 1937) have shown that while such is not the only effect of hybridization between species, it is certainly one of the commonest. We have therefore given it a distinctive name, introgressive hybridization. In discussing the effects of introgressive hybridization, we shall speak of the hybridization of one species into another rather than hybridization with another. This terminology is chosen as a matter of convenience in discussing particular cases and avoids needless repetition of explanatory phrases.

The application of the terms "hybrid" and "species" becomes a difficult problem in dealing with successive back-crosses between an original first generation hybrid and one of the parental species. The F_1 is clearly entitled to the term hybrid, but among the progeny of its first cross back to the parent there will be a number of individuals which resemble that species very closely indeed, and each successive back-cross will increase the percentage of these indistinguishable or almost indistinguishable mongrels. After a few back-crosses most of the individuals cannot be

distinguished by morphological means from the pure species, though even then a study of the group as a whole would indicate by its departure from the average of the species something of what had taken place. Further back-crossing would weaken even the effect upon the group average.

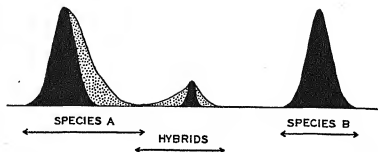


Fig. 1. Diagram illustrating the application of the terms "species" and "hybrids" to a case of introgressive hybridization. Solid black, original species and first generation hybrids. Dotted, later hybrid generations and back-crosses. Further explanation in the text.

Since it is therefore a practical impossibility to distinguish between individuals of partially hybrid ancestry and those of uncontaminated pedigree, we shall in the following discussion restrict the term hybrid to the more or less obvious intermediates between recognized species. The term species we shall use in a broad enough sense to include the barely perceptible variants, which may sometimes be of partially hybrid ancestry. Figure 1 illustrates the use of these terms in a case which, though hypothetical, is not unlike the relation between *T. canalculata* and *T. occidentalis*. The solid black indicates the two species 'A' and 'B' and their first generation hybrid. The stippled area shows the secondary hybrids (second generation and back cross to species 'A'). The arrows at the base of the figure designate the approximate limits of the terms "species" and "hybrids" as we shall use them. This use of terms is loose and somewhat illogical, but it avoids the difficulties which would attend an attempt at greater precision.

Anyone who has undertaken monographic work recognizes that there are slight regional differences within many species and varieties. Most taxonomists have been of the opinion that if such differences are too tenuous for cataloguing purposes, they should be ignored in taxonomic work, though they might very well be of considerable biological significance. A few botanists, like E. L. Greene or P. A. Rydberg, have advocated nomenclatorial recognition for such variants. These rather vague local or regional differences are common in some genera and rare or lacking in others. In the genus *Uvularia*, which the senior author has studied intensively (Anderson and Whit-

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aker, 1934), no such differences are perceptible, not even by the use of detailed biometrical methods. In the genus *Solidago*, on the other hand, the species in any one locality are usually comparatively stable, though fifty or one hundred miles away these same species may be significantly different. The causes of such differentiation are undoubtedly various and include such factors as the direct and selective effect of the environment, isolation, mutation, in addition to introgressive hybridization.

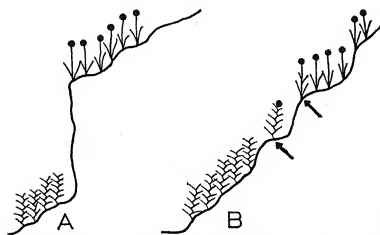


FIG. 2. Diagram illustrating the relation between the habitats occupied by *Tradescantia canaliculata* (above) and *T. subaspera* var. *typica* (below). A, above and below a cliff; B, in ravine at side of cliff, arrows point to back-cross hybrids. Further explanation in the text.

In the case of wide ranging and common species which have been extensively collected, it should be possible to obtain a rough estimate of the importance of such hybridization from a study of herbarium material. If, for instance, introgressive hybridization of *T. canaliculata* into *T. occidentalis* has been an important factor in producing regional differences in the latter species, then we should find that *T. occi-*

de be objectively scored, and (3) if there is enough herbarium material for the calculation of significant averages. Among the American *Tradescantias*, these conditions are met in three cases: (1) *T. subaspera typica* into *T. canaliculata*; (2) *T. canaliculata* into *T. occidentalis*; (3) *T. canaliculata* into *T. bracteata*.

TRADESCANTIA SUBASPERA VAR. *TYPICA* INTO *T. CANALICULATA*.—Throughout its range, which is entirely within that of *T. canaliculata*, *T. subaspera typica* tends to grow in strikingly different habitats from those occupied by the former species. In addition to this barrier there are differences in flowering season, and internal differences which cause the hybrids, once they have occurred, to be partially sterile. In spite of these various barriers, hybrids between the two species are to be found wherever the habitats come into contact without being sharply discontinuous. In much of the Ozark region, *Tradescantia canaliculata* is to be found growing in full sun at the top of cliffs, while *T. subaspera typica* is in deep shade in rich soil at the foot of the same cliffs. In this case the two habitats come into close contact, but with no intermediate zone, and consequently no hybrids. However, at those points where erosion has made a break in the face of the cliff, there are intermediate zones in which hybrids can nearly always be found. Figure 2 diagrams such a situation at Bat Cave, near Stanton, Franklin County, Missouri, which is typical of numerous other localities in the Ozarks.

When transplanted to experimental plots, the suspected hybrids have maintained their intermediate characteristics. Since they are partially sterile, and since they resemble very closely the artificial hybrids which have been raised between the two species (Anderson, 1936a), their hybrid nature can be taken as proved beyond a reasonable doubt.

TABLE 1. Comparisons of herbarium material of *TRADESCANTIA SUBASPERA* var. *TYPICA* marked "sub.", *T. CANALICULATA* outside the range of *T. SUBASPERA* marked "can.", and *T. CANALICULATA* within the range of *T. SUBASPERA* marked "can. (sub.)".

	Node number													Leaf number													Inter- node	Increase Decrease
	2	3	4	5	6	7	8	9	10	11	12	13		6	7	8	9	10	11	12	13	14	15	16	17	18		
Can.	2	12	15	15	12	5	3	1						3	10	22	12	10	2		2	3					50	15
Can. (sub.)			3	4	2										3	4	2										6	3
Sub.						1	1	2	5	2		1			2			3	4	2				1			12	

dentalis within and adjacent to the known range of *T. canaliculata* should be significantly different, on the average, from *T. occidentalis* outside that range. Furthermore the difference, however slight, should be in the direction of *T. canaliculata*.

Such a test can be applied only (1) if the distributions of the two species overlap only in part, (2) if there are a number of specific differences which can

The hybrids vary among themselves, some of them being obviously intermediate between the two species, others resembling one or the other parent more or less closely. Primary and secondary hybrids are therefore occurring at a large number of localities and presumably have been occurring for a considerable time. Is there any evidence that *T. subaspera* var. *typica* has introgressed into *T. canaliculata*?

The evidence on this point is assembled in table 1. It will be seen that there are three characters, readily scored on herbarium specimens, by which the two species differ. These are: (1) The number of evident nodes on the stem. *T. canaliculata* averages around five, while *subaspera* has about twice as many. (2) The number of leaves. Due to the fact that internodes may be so condensed at the apex and base of the stem, the number of leaves is higher than the number of apparent nodes. (3) In *T. subaspera* the upper internodes are shorter than the lower ones, usually decreasing in a harmonious sequence. In *T. canaliculata* there is no such sequence, and the upper internodes are often the longest.

When the specimens of *T. canaliculata* are assembled according to whether they are inside or outside the known range of *T. subaspera typica*, it will be seen (table 1) that there is little evidence for the introgression of *T. subaspera*. In spite of decisive differences between the two species, in all three cases the average difference between the two sets of *T. canaliculata* is very slight and of no statistical significance. Furthermore, in one case the direction of the differences is actually away from the value of *T. subaspera typica*.

specific races and strains, too vaguely defined for nomenclatorial recognition, but of great biological interest. It now becomes our purpose to examine the variation within *T. occidentalis* in detail and to learn if any portion of that variation may reasonably be assigned to the introgressive hybridization of *T. canaliculata*.

Tradescantia occidentalis var. *typica*, as defined by Anderson and Woodson (1935), is possessed of one strongly marked geographic race which is perhaps worthy of nomenclatorial recognition. It occupies the northwest corner of the range of that variety and differs from its fellows to the south and east by being less glaucous and more pubescent with wider bracts and larger sepals. It was to such plants that the names *T. laramiensi*s and *T. universitatis* were given, but the differences between them and *T. occidentalis* from Texas are slight, and they seem to intergrade completely. It has not been possible to separate them satisfactorily on the basis of herbarium work alone, though possibly extensive field work would yield critical evidence.

In so far as the relationship of *T. occidentalis* to *T. canaliculata* is concerned, these *laramiensi*s variants are of little or no importance. They occur well

TABLE 2. Comparisons of herbarium material of *T. CANALICULATA* marked "can.", *T. OCCIDENTALIS* var. *TYPICA* outside the range of *T. CANALICULATA* marked "occ.", *T. OCCIDENTALIS* within the range of *T. CANALICULATA* marked "occ. (can.)".

															Internode		Tuft					
	Node number								Leaf number								Increase	Decrease	None	Weak	Strong	
	2	3	4	5	6	7	8	9	6	7	8	9	10	11	12	13						14
Oec.		3	5						1	3	3	1						4	4	8	4	4
Oec. (can.) .		6	11	2					3	5	5	4	1	1				14	5	6	5	18
Can.	2	12	18	19	14	5	3	1	3	11	24	14	10	2		2	3	56	18		26	52

If there is introgressive hybridization of *T. subaspera* into *T. canaliculata*, it therefore is either so recent or so slight that it cannot be detected. The reverse introgression (*T. canaliculata* into *T. subaspera*) cannot be studied by this method, since the range of *T. subaspera* is entirely within that of *T. canaliculata*. This is unfortunate, since from various kinds of evidence we are led to suspect a strong introgression in that direction.

TRADESCANTIA CANALICULATA INTO *T. OCCIDENTALIS* VAR. *TYPICA*.—In more than one sense these two species are the counterparts of each other, the former in the middle west, the latter on the Great Plains. Each has a range of approximately 1,200,000 square miles. Each is clearly made up of two different elements: (1) truly indigenous strains and (2) ubiquitous weeds and plants of waste places, which, while they may be in part anciently established, are mostly post-Columbian. Each species is predominately tetraploid, though each has diploid races in certain restricted areas. Each species has numerous intra-

outside the area in which *T. occidentalis* and *T. canaliculata* commingle. Hence, their removal will not interfere with a study of the relationships of these two species.

Table 2, therefore, is a test of the introgressive effect of *T. canaliculata* in the southern and eastern parts of the range of *T. occidentalis* var. *typica*. While the numbers of specimens are too small to be particularly significant, it will be seen that the difference between the two sets of *T. occidentalis* in each of the four cases is in the direction of *T. canaliculata*. For the last two characters the differences are much more significant than mere numbers might indicate, since the difference is qualitative as well as quantitative. There is conceivably an almost endless series of forms and pubescence patterns which might occur in the genus *Tradescantia*. To find that within the range of *T. canaliculata* the only perceptible variation in the sepals of *T. occidentalis* is in the exact direction of *T. canaliculata* is most significant. These facts become even more illuminating when studied in con-

nection with the distribution of the two species (fig. 3). It will be seen that there is a steady increase in the percentage of *canaliculata* characteristics as one approaches the range of that species. If, for instance, we divide the range of *T. occidentalis* by the 90th, 95th, 100th, and 105th meridians, the percentages of specimens with tufts from west to east are 0, 25, 50, and 100 per cent.

To summarize, a study of herbarium specimens of *T. occidentalis* var. *typica* arranged with reference to the known range of *T. canaliculata* demonstrates the following points: (1) There is a slight difference between the specimens within the range of *T. canaliculata* and those from outside the range of *T. canaliculata*. (2) This difference, though slight, is in the direction of *T. canaliculata*. (3) There is some evidence that the intensity of the difference increases with the comparative frequency of *T. canaliculata*.

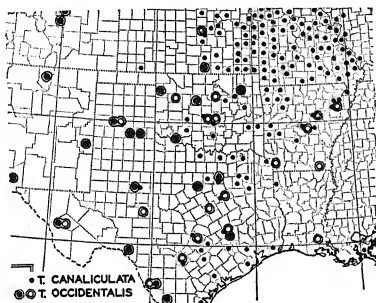


FIG. 3. Illustrating the relation between sepal pubescence in *T. occidentalis* var. *typica* and the distribution of *T. canaliculata*. The white centered dots represent specimens of *T. occidentalis* var. *typica* with barbate eglandular hairs on the sepals.

From these three points and from the known fact that *T. occidentalis* and *T. canaliculata* cross readily, we conclude that there is a strong introgression of *T. canaliculata* into *T. occidentalis*. This conclusion receives confirmation from such field studies as it has been possible to make of the variation within *T. occidentalis*.

We may anticipate the evidence given in detail below by saying that variation in natural population of *T. occidentalis* is proportional to (1) the weediness of the environment, (2) the number of other species of *Tradescantia* in the neighborhood, and (3) the comparative frequency of other species of *Tradescantia*.

If introgressive hybridization takes place, we should expect to find the strongest evidence for it in those regions and at those points where there is greatest opportunity for hybridization. The greater the number of closely related species in the vicinity, the

greater the chance that at least one of them will come into effective contact with the species being studied. Similarly, the greater the frequency of any one of these foreign species the greater is the chance that crossing may take place. While the connection between weediness and introgressive hybridization may not be so transparent, it is even more important (Wiegand, 1935). In the genus *Tradescantia* one of the chief barriers between closely related species is differences in habitat preference. When a railroad is constructed, or a roadway, or ditches, or when in various other ways man disturbs the ecological complexion of the countryside, these barriers are broken down. Species which before were kept apart now grow side by side, and what is even more important, a whole set of various new intermediate environments is provided in which hybrids may establish themselves. We should therefore predict that the more that natural ecological conditions have been upset the greater would be the opportunity for introgressive hybridization. The analysis of variation in the following natural populations proves this to be the case.

Mirando.—These plants were growing near Mirando, Webb County, Texas in typical mesquite shrubland. The land had been fenced for livestock and had been grazed, but natural conditions had not been greatly altered. Although some of the plants were growing between the fence and the highway, there was no evidence that they had been brought in by road construction. No other species of *Tradescantia* are known from the immediate neighborhood. Twenty plants were found in bloom and collected.

Detailed scoring of certain features is presented for ten of these in table 3, and summaries for the entire group are shown in table 4. As the table indicates, there is little variation from plant to plant, and in such characters as have been used for specific delimitation, there is none at all.

Rainbow Bridge.—For this collection we are indebted to Hugh C. Cutler who collected it near Rainbow Bridge, San Juan County, Utah. It grows there in fairly stable sands throughout Bridge Canyon. The spot is remote from highways, and grazing animals are few. It may be seen from table 4 that there is little variation between the twenty-one plants which were studied and that they resemble the plants from Mirando.

Inscription House.—This collection was also made by Mr. Cutler. The population of which this is a sample was growing in deep sand on a slowly moving dune, eleven miles north of Inscription House Post, Navajo County, Arizona. The spot was 300-400 yards west of the road leading to Rainbow Lodge. Although the region is practically a wilderness area, the roadway is an ancient one, having originally been an Indian trail. The plants are, for the most part, fairly uniform and resemble those from Rainbow Bridge very closely. Unlike that collection, however, about a third of the plants show eglandular tufts on the sepals and have glabrous pedicels. These char-

acters, in our opinion, can be explained only as due to introgression from *T. canaliculata*. If this be true, it demonstrates how extremely difficult, in such a species as *T. occidentalis*, is the determination of the exact extent to which introgressive hybridization is due to man's interference.

Norman.—This collection consists of thirty-two plants collected along the dirt roadway at Indian Springs, four miles south of Norman, Cleveland County, Oklahoma. Some of the plants were growing in the actual roadway, and the whole population was evidently spreading along the road. There are no other species of *Tradescantia* in the immediate neighborhood, although *Tradescantia canaliculata* is known to occur a few miles away. All the plants possessed the typical glandular, sepal hairs of *T. occidentalis*, although a quarter of them were glabrous on the pedicels, or nearly so. Furthermore, three plants had eglandular tufts on the sepals. Both of these are characteristics of *T. canaliculata*, and the eglandular tufts are unique in that species.

College Station.—These plants were collected along a railroad right-of-way south of College Station, Brazos County, Texas, and in the door-yards of negro cabins adjacent to the railroad. A much larger collection was made but was unfortunately lost before detailed records were made. However, we know from a study of it that these few plants are typical of the lot as a whole. These eleven were cultivated in the greenhouses of the Bussey Institution for several years and retained their distinctive characteristics throughout that period. All the plants exhibit the typical glandular hairs of *T. occidentalis*, although a majority of them show an eglandular tuft at the apex, like *T. canaliculata*. This latter species has been collected at various nearby points. Particularly significant is the fact that it has been found several miles farther north, along the same railroad right-of-way.

Scott-Dusan.—These forty-eight plants are from a large colony which extends more or less continuously along the railroad right-of-way between Scott and Dusan, Lafayette County, Louisiana. At the eastern end of the colony there are a number of plants of *T. hirsutiflora*, a variable weed along the gulf coastal plain. As its name indicates, the calyx is more or less covered with long, scattered, eglandular hairs. It hybridizes more or less extensively with *T. canaliculata* and with various other species of *Tradescantia*. Of the plants collected at Scott-Dusan the majority were either typical of *T. occidentalis* or differed only by a slight eglandular tuft at the apex. There were a few clear-cut plants of *T. hirsutiflora* and a complete set of intergrades between *T. hirsutiflora* and *T. occidentalis*.

Comal County.—This collection of thirty-seven plants was made on the edge of the Edwards escarpment along the highway running west from New Braunfels, Texas. In table 3 the detailed scoring of ten plants is presented in contrast to a similar ten from Mirando, Texas. The Comal County population had almost the maximum chance for contamination.

In the first place, Comal County is in the very center of diversity for the American species of *Tradescantia*. In the second place, the collection was made along a roadside ditch where there had been repeated grading, travelling, and other alterations of the original terrain.

TABLE 3. Comparative variation between 10 plants of *T. OCCIDENTALIS* var. *TYPICA* in "weedy" (Comal Co.) and "non-weedy" (Mirando) environments.

Locality	Glandular hairs on pedicels	Length of pedicel hairs	Length of sepals (in flower)	Glandular hairs on sepals	Eglandular tuft on tip of sepals	Eglandular hairs scattered over sepals
Mirando, Texas	×	4	6	×		
	×	4	6	×		
	×	3	6	×		
	×	5	6	×		
	×	4	6	×		
	×	3	6	×		
	×	3	5	×		
	×	4	6	×		
	×	3	7	×		
	×	3	5	×		
Comal Co., Texas		.0	11	×	×	×
	×	4	8	×	×	
	×	5	8	×	×	×
	×	4	9	×		×
		.0	9	×		×
		.0	9	×		
	×	4	9	×	×	
	×	2	11	×	×	×
	×	4	12	×	×	×
	×	3	10	×	×	×

Two miles eastward, on the edge of the escarpment, *T. gigantea* spreads down from the cliffs to the side of the same road. Under *Opuntia* in neighboring pastures there were extensive colonies of *T. humilis*, a low-growing, hairy species. The plants along the roadside, on the whole, were more like *T. occidentalis* than like any other species. The majority of them, encountered singly in the herbarium, would have been classified as belonging to that species or to have resulted from hybridization with it. One or two plants were morphologically almost like *T. canaliculata*; others showed extensive contamination with *T. humilis*, and for the most part these particular intermediates were growing at the edge of the roadway, on the side towards the adjoining pastures.

Of particular significance was the fact that the Comal County plants when examined cytologically proved to be tetraploids, though *T. occidentalis*, as well as all other native species of *Tradescantia*, is

usually diploid in central Texas (Anderson and Sax, 1936; Anderson, unpublished). In *Tradescantia*, as in many of the higher plants, tetraploids tend to be more ubiquitous and to have more extensive distributions than the diploids from which they arose (Müntzing, 1936).

TABLE 4. Summaries of seven populations of *T. OCCIDENTALIS* var. *TYPICA* arranged according to the magnitude of introgression.

	Total number of specimens	Glandular hairs on pedicels	Glandular hairs on sepals	Eglandular tuft on tip of sepals	Eglandular hairs scattered over sepals
Mirando, Texas	20	100%	100%	0%	0%
Rainbow Bridge, Utah .	21	100	100	0	0
Inscription House, Ariz.	30	73	100	63	0
Norman, Okla.	32	25	100	12	0
College Station, Texas .	11	100	100	82	0
Scott-Duncan, La.	48	81	92	94	27
Comal Co., Texas	37	73	97	78	57

From the mere fact of tetraploidy we would have predicted that the Comal County collection was not a part of the indigenous *Tradescantias* of the region, but was in some way derivative. It was the only tetraploid found during two weeks of collecting in central Texas. It was also the "weediest" population in that area.

TABLE 5. Comparisons of herbarium material of *T. CANALICULATA* marked "can.", *T. BRACTEATA* outside the range of *T. CANALICULATA* marked "bract.", and *T. BRACTEATA* within the range of *T. CANALICULATA* marked "bract. (can.)".

	Node number									Leaf number								Internode			Tuft		
	2	3	4	5	6	7	8	9		5	6	7	8	9	10	11	12	Increase	Decrease		None	Weak	Strong
Bract.	11	1								1	7	3	1					6	6		4	7	1
Bract. (Can.) ..	2	9	9	2						1	8	8	4	1				12	10		1	3	19
Can.	2	12	18	19	14	5	3	1		3	10	22	12	10	2		2	56	18			26	42

TRADESCANTIA CANALICULATA INTO *T. BRACTEATA*.—*Tradescantia bracteata* is confined to the middle-western prairies and to the northeastern edge of the great plains. Under natural conditions it is typically found in rich soil at the edge of prairie swales. Under cultivation it persists along the edge of drainage ditches and may become very abundant along roadways or railroads if they pass through an area with rich soil and a high water table. In such situations it comes into close contact with *T. canaliculata*, which is almost universally present along railroad rights-of-

way in the middle west. Although *T. bracteata* is a diploid and *T. canaliculata* prevalently tetraploid (Anderson and Sax, 1936), and although *T. bracteata*, like most diploids, has a short blooming period, hybridization between the two species does occasionally take place, and from the variation in such populations it is evident that the hybrids must be partially fertile.

Table 5 shows that there is apparently an introgression of *T. canaliculata* into *T. bracteata*. In every case the average of the difference is in the direction to indicate introgression and the numbers are large enough to be fairly significant. The evidence is somewhat weakened, however, by the peculiar status of *T. bracteata*. This species, quite aside from any possible introgression of *T. canaliculata*, presents the most complicated pattern of intra-specific variability which has yet been encountered among the American *Tradescantias*. Until this phenomenon has been studied exhaustively, the evidence for an introgression by *T. canaliculata* will be less conclusive.

CONCLUSIONS. — *Taxonomic*. — The morphological effect of introgression in these species is too slight to merit nomenclatorial recognition. While it may be of interest to taxonomists, introgression is here of slight taxonomic significance.

The evolutionary importance of introgressive hybridization.—In genera such as *Tradescantia* the presence of introgressive hybridization greatly complicates the study of the evolutionary dynamics of populations. Were such genera as *Tradescantia* rare, evidence like that presented above could be dismissed as rather a special case. There are, however, a very large number of genera of the flowering plants which resemble *Tradescantia* in that closely related species

are separated largely by ecological barriers (Wiegand, 1935). In such genera it will require exceedingly critical data to demonstrate (1) the relative importance of hybridization and mutation and (2) the relative importance of introgressive hybridization in weed populations and under strictly natural conditions.

In most of the populations described above, the effect of introgressive hybridization was so overwhelming that the effect of such presumably more basic evolutionary factors as gene mutation was completely obscured. Much of the introgression in these

populations is post-Columbian. To estimate the role which introgression may have played before the ecological complex of the Great Plains was catastrophically altered by the Caucasian invasion will require detailed analyses of many strictly indigenous populations. It is to be hoped that such populations may be found.

In the last decade a number of techniques, chiefly cytological, have made possible the precise exploration of the germplasm. Such pioneer work as that of Dobzhansky and Sturtevant (1933) is demonstrating the precision which these discoveries bring to the study of phylogeny. Although, in the higher plants, the microstructure of the germplasm cannot yet be determined by direct exploration, as in the diptera studied by Dobzhansky and Sturtevant, much pertinent information can be obtained by inference (Darlington, 1937; Upcott, 1937). These methods are of great promise for the determination of the evolutionary importance of such factors as segmental inversion. Unfortunately, in genera like *Tradescantia*, the importance of inversion cannot be estimated, much less demonstrated, without an understanding of the nature and degree of introgressive hybridization.

In such genera the cytological complex of the population is a reflection of the ecological complex of the environment. Ecological data are therefore fundamental to the interpretation of cytological data. Refined cytological analyses of the germplasm will require equally refined ecological analyses of the environment before the potentialities of the data can be realized.

SUMMARY

Introgressive hybridization is described and defined.

Analyses of intraspecific variation in herbarium material indicate a strong introgression of *Tradescantia canaliculata* into *T. occidentalis* and of *T. canaliculata* into *T. bracteata*. There is no evidence for an introgression of *T. subaspera* into *T. canaliculata*, although these species are known to hybridize.

The above conclusions are supported by an analysis of variation in natural populations of *T. occidentalis*. This analysis further demonstrates that introgression is roughly proportional to the frequency of the introgressive species and that it is greater when plants are growing as weeds than when they occupy more natural habitats.

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THE COLLOIDAL CLAY FRACTION OF SOIL AS A CULTURAL MEDIUM¹

Wm. A. Albrecht and T. M. McCalla

A BETTER understanding of the properties and behavior of colloidal clay makes it now possible to use this fraction of the soil in plant nutrition studies as a means of obtaining a closer approach to complete chemical control of the soil factor in plant growth. Though the soil may be more than a mixture of mineral particles of sand, silt, and clay sizes as a consequence of their possible chemical linkage (Sideri, 1937), yet the sand and silt separates are too large to offer much active surface. The surface activity for the adsorption of mineral nutrients and their delivery to the plant by ion exchange (Jenny and Cowan, 1933; McCalla, 1937) must then rest with the colloidal clay fraction. Such activity in the clay fraction of the Putnam silt loam, for example, suggests a supply of nutrients one hundred times as large as that in the leachings from the lysimeters at Cornell (Lyon and Bizzell, 1921). Recent experimental use of colloidal clay as a growth medium for soil microorganisms and for leguminous plants suggests a wide range in its adaptability to well controlled nutritional experiments (Albrecht, 1932, 1933); Albrecht and Jenny, 1931; Albrecht and McCalla, 1937), aiming at a nearer duplication of soil conditions than has previously been possible.

The low concentration of soil solutions and irregularities in plant growth in aqueous media have suggested that the soil provides the plant from sources other than simple solution conditions. Hoagland and Martin (1923) suggested that the soil medium was more productive than aqueous solutions when they said, "In several experiments we have found the average weight of the kernels produced in solution cultures about 80 per cent of the weight of the kernels produced in a good soil." Jones and Pember (1925) observed less plump kernels of grain for the solution cultures than for the soil, and also a wider ratio of straw to grain. Some workers (McCalla, 1916; Breazeale, 1906) ventured the idea that the addition of solid material to a solution culture may result in the adsorption of toxic substances by the solid and thus increase plant growth. Jennings (1919) found that the total plant growth was increased by the addition of colloidal silica to the cultural solution. The effect was attributed to the silica taken by the plant. Haley's (1923) data, showing increased plant growth from the addition of 75 grams of finely ground orthoclase to the solution, could not be attributed to this as a supply of potassium since the plants in the aqueous solution alone contained the more potassium. MacIntire, Shaw, and Young (1925) found silica lessening the injurious effects by magnesium on plant growth with increasing benefits as more silica was used. Shive (1919, 1920) using fine sand, concluded that this was without effect on the nutrient solution

when the sand was freed of possible colloidal matter. Barley and peas grown together in a control nutrient solution and in an adobe soil showed differences in the amounts of minerals taken under these conditions, according to Newton (1923). Greaves and Pulley (1931) found nitrogen fixation greater in soil than in solution cultures. "The environmental conditions, affecting the absorption of elements from the soil," according to Thomas (1932), "are different from those under which nutrient culture experiments are conducted because: (a) the rate of diffusion of ions toward the root zone is more rapid in nutrient culture solution than in the soil, and (b) the extent of the root system, and therefore the absorbing surface, is greater under field conditions than in nutrient culture solution." Ion exchange between the soil and the plant was suggested for phosphorus by Hoagland (1931), since the soil solution seldom contains much phosphorus even under good plant yields (Hoagland and Martin, 1923). "For the phosphate nutrition of plants," Parker and Pierre (1928) believe that "root-soil contact is necessary."

The importance of the clay fraction as a carrier of the supply of nutrients came to be appreciated as a consequence of the ability of plants to take from the soil much larger quantities of nutrients than any soil solution test ever indicated present there. With fuller understanding of the adsorbed ion supply on the clay and the exchangeability thereof, the clay fraction as the carrier of the essential nutrients becomes the significant part of the soil for the soil microbiologist, plant physiologist, and the agronomist.

EXPERIMENTAL STUDIES USING COLLOIDAL CLAY MEDIA.—Recent studies have shown that the clay fraction of the soil may serve as a source of nutrients. As evidence of this possibility, experiments carried out to date using nutrients adsorbed on the clay and thus not water soluble show that such can be (a) removed by soil microorganisms using them as a source of energy, as the ammonium ion, for example, is oxidized to nitrates; (b) used by soil microorganisms in their regular metabolic processes for growth, as calcium, for example, is taken by legume bacteria (Rhizobia); and (c) taken up by plants for their regular growth processes in quantities related to the supply which is determined either by the total clay or by the degree of saturation of the clay with the respective nutrient ions.

For soil microorganisms (*Nitrosomonas*).—Colloidal clay with only adsorbed ions and no water-soluble, or solution ions, present can serve as a medium for the soil microorganisms bringing about nitrification or for the change of the adsorbed ammonium ion to the soluble, non-adsorbed nitrite ion. As a test of this possible change of adsorbed ammonia, a colloidal clay medium was prepared as follows: Hydrogen clay, or electrodialyzed colloidal clay (Bradfield, 1923, 1928),

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with a pH 3.6₂ was treated with magnesium oxide at the rate of 4 milligram equivalents (M.E.) per 100 grams of clay—or 1 M.E. per liter containing 25 grams of clay—and allowed to react with occasional stirring for two days. Dipotassium phosphate was next added in an amount required to supply 4 M.E. of the phosphate ion and 2.66 M.E. of potassium per liter. After some time had elapsed to permit the reaction to take place, the suspension was then put through a collodion-coated filter paper and washed in the ultra filter under pressure of 100 pounds per square inch. The clear filtrate carried no cations but removed slightly more than 9 per cent of the added phosphate anion. The resuspended clay with a pH

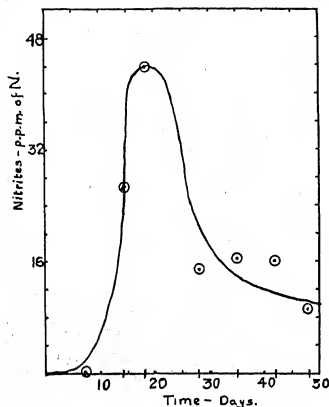


Fig. 1. Accumulation of nitric nitrogen resulting from the conversion of adsorbed ammonia on the colloidal clay in suspension.

of 4.55 had 86 per cent of the added phosphate in electro-dialyzable condition and 83 per cent available by the Truog (1930) test. It was then given calcium as the hydroxide in an amount of 6 M.E. per liter with the pH value 5.2 resulting. The medium so prepared served not only for nitrification by soil microorganisms but needed only slight alterations to serve as a growth medium for the legume bacteria (*Rhizobia*). As the source of nitrifiable material, ammonium hydroxide equivalent of 7 M.E. per liter was then added to bring the final pH value to 7.0. Thus a neutral clay resulted without water-soluble ions, but with adsorbed cations of magnesium, potassium, calcium and ammonium, and the anion of phosphate present in these different amounts.

This clay suspension, when sterilized and inoculated with nitrifying organisms, served as a medium for these microorganisms. When clarified of the clay,

²The hydrogen-ion concentration was measured by means of the glass electrode.

either by ultra filtration or by potassium chloride addition and centrifuging at regular time intervals, it showed an increase in its content of nitrites for about two weeks. This was then followed by a decrease, as is shown in figure 1, when there was evidence of the production of nitrates by the possible conversion of the nitrites into this form of more completely oxidized nitrogen. The increase in nitrates was not commensurate with the decrease in nitrites.

Though the fate of the nitrite nitrogen was not followed with mathematical precision in this study, the fact was clearly demonstrated that with the adsorbed ammonium cation as the only more or less immobile form of nitrogen present at the outset, there resulted a decided accumulation of this element in the readily soluble, nitrite, anion form to a concentration amounting to about 45 per cent of that originally adsorbed. At the same time, the originally neutral clay became more acid. This occurred to such an extent that the clay lost more than two-thirds of its original cation saturation for hydrogen. The study suggests that not only the ammonium ion, but also other nutrient ions were released from the clay through hydrogen substitution for them. It points forcefully to the function of colloidal clay as a source of energy and growth nutrients for these soil microorganisms.

For legume bacteria (Rhizobia).—The nodule producing bacteria (*Rhizobia*) occasionally change from their normal, clear, watery, viscous, raised colonies on agar to a yellow, or red, or a form which is quite abnormal. It seems probable that this variation is associated with a failure of the microorganism to obtain sufficient calcium. Even though Ashby's medium

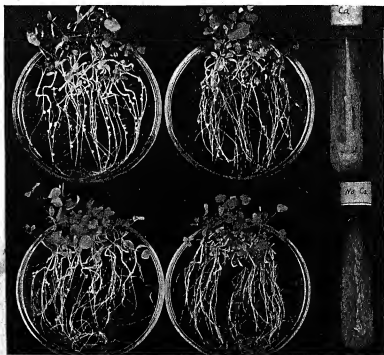


Fig. 2. Growth on agar slants, and nodule production as a result of inoculation, by an abnormal (below) *Rhizobium* culture given no calcium, and by the same restored to normal when given adsorbed calcium liberally. Plants on left were given calcium, on right no calcium during growth.

as commonly used for *Rhizobium* growth in the laboratory is saturated with calcium carbonate, it contains per liter only 1.3 M.E. of soluble calcium (Albrecht and McCalla, 1937; McCalla, 1937).



Fig. 3. Growth variation with increasing degree of calcium saturation of clay (left to right) and constant amounts of adsorbed calcium. This variation occurs regardless of variation in acidity by hydrogen presence, or of neutrality and of variable barium supply.

Since the colloidal clay medium cited above will provide much more calcium in the adsorbed form than this, it was decided to test whether the abnormal characters of *Rhizobium* occurring on agar medium could be restored to normal characters by growth in a medium offering sugar and a clay suspension supplying the adsorbed nutrient cations with calcium in abundance. Growth trials of inoculation from yellow colonies into the clay and sugar medium restored these abnormal forms to normal appearance when plated out again on clear agar. Such abnormal forms originally unable to inoculate the host were likewise restored in their inoculating ability and served more effectively when the host plant was amply provided with a liberal supply of calcium (McCalla, 1937), as is shown in figure 2.

Changes in the reaction of the colloidal clay toward increased acidity indicated the cation removal by the microorganisms. These data point out that such a medium served to provide these bacteria with the cation nutrients and in more effective manner than was true for the more common laboratory agar medium. Growth activity in the calcium clay was many times more rapid than in nutrient solutions saturated with ordinary calcium carbonate, and served as the suggestion for an improved *Rhizobium* medium using calcium gluconate instead (Albrecht and McCalla, 1938).

For plant growth.—Colloidal clay with its adsorbed nutrient ions serves as a medium for plant growth much as it serves for microorganisms (Albrecht and Jenny, 1931; Horner, 1936; Hutchings, 1936). Trials with variable supplies of calcium (Albrecht, 1932), of magnesium, and of potassium (Horner, 1936), adsorbed on clay mixed with quartz sand, have been

conducted to date and indicate the possibility of a wide range in the use of this method of controlling the nutrient supply offered the plant. Colloidal clay with a known supply of adsorbed ions used in definite amounts of these per plant provides a wide possibility for control of the soil factor. Accurate analysis of the corresponding seed at the outset and of the resulting plant and the clay after plant growth permit an understanding of ion relation to growth and the interrelations of different ions not yet well understood.

The variable supplies of a cation per plant, for example, may be provided either by offering the plant different amounts of a saturated clay or by offering different amounts of clay with different degrees of saturation (Albrecht, 1932). Differences in plant growth under such test using calcium, for example, show clearly, as in figure 3, that calcium is more effectively delivered to plants from more completely saturated clays. Also, an increased content in the soil of clay at any degree of saturation can serve more effectively in providing this element, as shown in figure 4 (Horner, 1936). Tests with variable supplies of magnesium, as illustrated in figure 5, point to a similar behavior of the clay soil fraction. Further, not only cations are adsorbed, but the anion of phos-

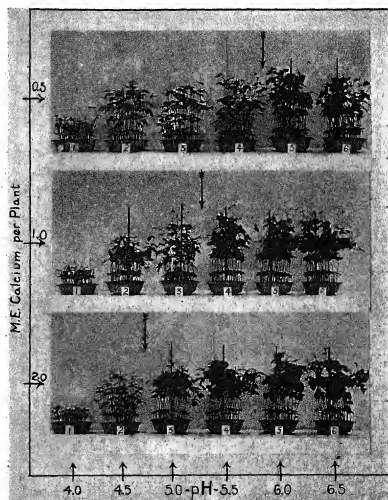


Fig. 4. Improved growth of soy beans with increased clay content of the sand regardless of its pH. Middle row had twice as much clay and lower had four times as much as the upper row. (The effects of pH are lessened by increased clay content offering more calcium).

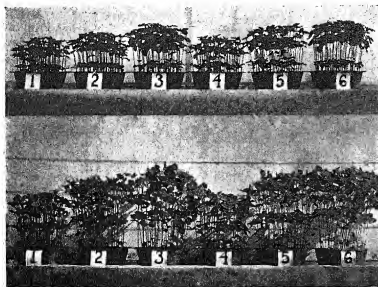


Fig. 5. Growth variation in soy beans in response to increase in adsorbed magnesium on colloidal clay in sand. (Increases in magnesium from 1 to 3 and 4 to 6. Lower row a later photograph of upper).

phate is also taken by the clay fraction and changed into an adsorbed form which is not water-soluble but yet is electrodialyzable and usable by plants (Hutchings, 1936). Possibly other anions will later be found to function similarly.

SUMMARY

These preliminary trials suggest a large field of study opened by the use of colloidal clay under controlled, adsorbed, ion supply. It provides a means whereby more complete chemical analyses of the soil and seed at the outset and soil and plant at the close are possible with an accuracy and interrelation of results never before possible in such high degree of approach to actual soil conditions. Studies to date indicate equilibrium conditions as to cation movement between the plant and the clay at certain degrees of saturation by the hydrogen ion, for example, at which the plant fails to absorb more nutrients from the soil. Colloidal clay deficient in nutrient cations has even taken these from the growing plant which finally contained less of these than were present in the seed at the outset. Physico-chemical studies of the changes by colloidal clay in consequence of plant growth offer possibilities of knowledge of soil behavior never conceived possible in the complex mixture of sand, silt, and clay as soil. These bid fair to contribute much to our more complete understanding of plant nutrition in relation to soil conditions and to the soil as a nutrient source for crop production.

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THE MORPHOLOGY OF THE FLOWERS OF THE JUGLANDACEAE.

I. THE INFLORESCENCE¹

Wayne E. Manning

THE JUGLANDACEAE and other families of the Amentiferae have been considered primitive by many botanists because of their long geological history, their simple floral structure, and their cone-like inflorescences. Recently the evolutionary position of the Amentiferae has been questioned, and a few families—*Salicaceae* (Fisher, 1928) and *Betulaceae* (Abbe, 1935)—have been investigated to determine whether their simple structure is a primitive feature or the result of reduction accompanying specialization for wind pollination.

The walnut family itself has long been of special interest, and many investigations have been made of the vegetative and the floral structure of its members. The more extensive papers on the floral structure are by DeCandolle (1862), Eichler (1878), Engler (1889), Nicoloff (1904-1905), and Nagel (1914). The first three give a survey of the characteristics, including the inflorescence, of all genera of the family; Nicoloff deals primarily with the structure of the individual flowers; Nagel surveys the vegetative and floral characteristics, reviewing in detail the fossil forms. But the exact nature of the inflorescence and of the parts of the flower is still in question, and there is decided confusion evident in the varying descriptions of the family in different modern books, especially in American ones. Mistakes in the early works, the scarcity

of illustrations, the insufficient stressing of some important species, and the recent discovery of one genus and several species, make it seem wise to undertake a new and more thorough investigation of the floral morphology of all the genera and of most of the species of the family and to illustrate as many of these as possible. Furthermore, very few attempts have been made in the *Juglandaceae* to determine the origin of the catkin and the evolutionary position of the genera within the family. All progress in these lines should help to determine the ancestry of the family.

This paper deals with the structure and evolution of the inflorescence in the family; later papers will deal with the structure of the staminate and of the pistillate flowers.

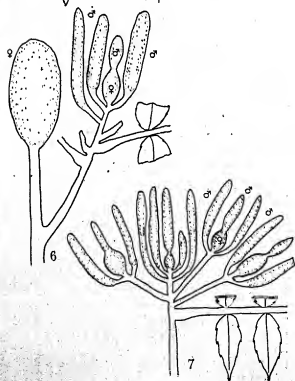
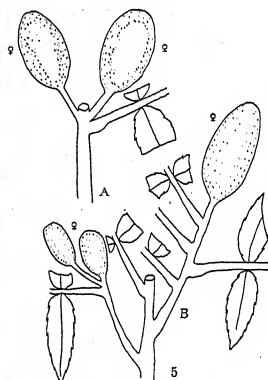
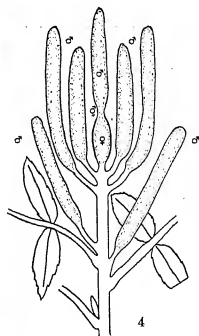
DESCRIPTION.—Eight genera—i.e., *Platycarya*, *Engelhardtia*, *Oreomunnea*, *Alfaroa*, *Pterocarya*, *Juglans*, *Waltia*, and *Carya*—have been recognized in recent books. As will be explained in a later paper, *Oreomunnea* should be combined with *Engelhardtia*, and *Waltia* with *Juglans*. This leaves six quite distinct genera. At least fifty-four species are now known.

The flowers are mostly imperfect, and the trees are usually monoecious, but in a few species of *Engelhardtia* they are evidently dioecious. The arrangement of both staminate and pistillate flowers in elongated spikes or racemes, "catkins," is well known. The individuality of the catkin is emphasized by most writers, but in several of the genera the staminate catkins, at least, are clustered together in a panicle-like inflorescence; in species of some genera, too, the staminate and pistillate catkins occur in the same cluster, forming an androgynous inflorescence. In this paper the term inflorescence refers to the complete flower cluster, whether this consists of several catkins or of a solitary catkin. The inflorescences containing pistillate flowers are mostly terminal, staminate inflorescences usually lateral. In many species the lateral inflorescences are superposed in relation to vegetative buds or branches, or even to other inflorescences.

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The grouping of the catkins into the cluster or inflorescence and the location of the inflorescences on the twigs will be described under each genus. The descriptions have been drawn from living material and from herbarium specimens of the principal American herbaria. They will include not only the typical condition but also the more important variations. These descriptions will form a foundation for the discussion of the evolutionary trends of the inflorescence in the family.

Platycarya (1 species, *P. strobilacea*).—The inflorescence (fig. 1, 2, 39A) is a panicle, consisting of a compact cluster of four to nine densely flowered spikes attached to a common naked basal rachis; the central spike or catkin is largely pistillate; the other catkins of the cluster surrounding this are staminate, each of these short-stalked in the axil of a deciduous scale, or often of a foliage leaf. The central catkin, pistillate below, is usually terminated by a staminate tip, commonly short but sometimes elongated; at the point of union of the pistillate and staminate parts, the flowers are small and perfect though usually abnormal in some respect. Occasionally the pistillate catkin aborts, and the inflorescence is a staminate panicle (fig. 39B). Staminate catkins are early deciduous (see fig. 3, 5, 6).

The staminate as well as the pistillate spikes are erect, whereas the catkins of the other genera in the family usually droop. The pistillate flowers are very numerous and densely crowded, the bracts of the individual flowers rather conspicuous and woody, and the fruiting catkin very closely resembles a cone (fig. 3).

In *Platycarya* the inflorescence is typically terminal at the ends of the present year's main shoots (fig. 39). There may be additional lateral inflorescences in the axils of the upper leaves (fig. 2, 3, 6), or if the tip of the stem dies, one to several lateral inflorescences may replace the normal terminal one (fig. 5), but these conditions are rare. The lateral inflorescences are definitely stalked, the stalk usually bearing one to several small leaves. Thus these panicles are really terminal on short or long branches. Two or more panicles may rarely be clustered at the tip of the stem forming a "compound" panicle (fig. 7).

Separate staminate catkins often occur in the axils of leaves just below a normal terminal inflorescence (fig. 4). Each catkin may be a distinct sessile axillary inflorescence (this is the interpretation of DeCandolle (1862)), in that case reduced from a complete axillary branch panicle such as the one discussed above. These catkins, however, are probably a part of a terminal inflorescence in which the lower inter-

nodes are much elongated. DeCandolle intimates also that the pistillate catkin may be solitary and axillary; this is an erroneous interpretation, based on older inflorescences from which the staminate catkins have dropped off (cf. fig. 2, 3, 5). In *Platycarya* the cluster of catkins is a unit.

The pistillate catkin is always terminal in the panicle, and the staminate ones axillary, except the one terminating the pistillate catkin.

Engelhardtia (11 species).—The variation in the type and position of the inflorescence in this genus is unusually diverse. The variation is best shown by discussing the condition in representative species.

The inflorescence of *Engelhardtia chrysolepis* (*E. Wallichiana*, *E. formosana*, and *E. fenzlii* are synonyms) is very important and significant. It is not clearly described by De Candolle, Eichler, or Engler. In this species, as in *Platycarya*, the typical inflorescence is an androgynous panicle, terminal on the main shoots (fig. 8, 18, 41A). It consists of a central pistillate catkin surrounded by several staminate ones. The inflorescence of *E. chrysolepis* differs in minor respects from that of *Platycarya*: the pistillate catkin is always completely pistillate and is occasionally attached at the side of the staminate ones; all catkins are drooping, longer, more slender and more loosely flowered; the inflorescence bracts at the base of the catkins are rarely foliar, and the common rachis of the panicle is always short. This typical androgynous inflorescence is often reduced by abortion to a staminate panicle (fig. 13, 14) or to a solitary pistillate catkin (fig. 12, 41B); these two or three types of inflorescences may occur on one tree. The trees are monoecious, but have been reported as dioecious as well. Nagel (1914) divides the species into two, *E. Wallichiana* being dioecious, *E. chrysolepis* monoecious. The writer believes that there is only one species, which is normally monoecious. In fruiting condition the inflorescences of a tree may all appear to be pistillate, but these may have been androgynous when in flower, the staminate catkins falling off early. In most cases there are scars of such catkins at the base of the fruiting catkin (x, fig. 17).

In *Engelhardtia chrysolepis* the inflorescence is usually terminal at the tips of the main shoots. As in *Platycarya*, additional inflorescences may be terminal on leafy branches from this season's growth (fig. 8, 18). These branches are in some cases reduced in length, and the panicle may seem axillary. In this species, as in most species of *Engelhardtia*, there is little distinction between new and old wood; consequently it is difficult to tell whether some of the

Fig. 1-7. *Platycarya strobilacea*.—Fig. 1-3. Photographs of different twigs of tree at Brooklyn Botanic Garden.—Fig. 1. Detail of terminal panicle; the staminate flowers at the tip of the central catkin are not yet open.—Fig. 2. Twig with panicles terminal on twig and on short side branches.—Fig. 3. A similar twig in fruiting condition (courtesy Brooklyn Botanic Garden).—Fig. 4-7. Outline sketches, showing deviations in the type and position of the inflorescences.—Fig. 4. Elongated terminal panicle; note the long staminate tip of central catkin, and the stalks of the staminate catkins.—Fig. 5A, B. Twigs in fruiting condition; tip of twig broken off.—Fig. 6. Fruiting catkin at tip of twig and flowering panicle at tip of long side branch.—Fig. 7. Compound panicle, made up of several individual panicles (from herbarium specimen labelled, *Flora Japonica*, 12/6/1903).



Fig. 8-11. Flowering twigs. Fig. 8-10, $\times \frac{1}{4}$; fig. 11, $\times \frac{1}{2}$. Reference is made to only a few of the herbarium specimens from which drawings were made.—Fig. 8. *Engelhardtia chrysolepis* (from Ching no. 1980 and Henry no. 13049).—Fig. 9. *Engelhardtia (Oreomunnea) pterocarpa*: A, staminate catkins not yet elongated; B, inflorescence in full flower (drawn from photograph of herbarium specimen at Geneva).—Fig. 10. *Alfaroa costaricensis*.—Fig. 11. *A. Juglans cinerea*: b, inflorescence bract; B, *J. nigra*, pistillate catkin 2-flowered; C, *J. Sieboldiana*, pistillate catkin 12-flowered.

lower branches of a twig, long or short, which bear inflorescences at their tips, grow from new or from old wood.

Figures 14 to 17 show deviations in the arrangement of the inflorescences that are not well represented in herbaria. Two or more panicles, some of them staminate, some androgynous, are clustered together, forming a compound panicle near the tip of the stem. The excellent illustration labelled *E. Roxburghiana* in Wallich (1831) is of a compound panicle of *E. chrysolepis*. In older, fruiting stages (fig. 16, 17), after the staminate catkins have fallen off, it is difficult to tell whether a compound panicle was originally present or whether there were two or more separate panicles.

In the other species of *Engelhardtia* the catkins are thicker and more densely flowered, and the common rachises of the panicles are usually elongated. In *Engelhardtia (Oreomunnea) pterocarpa* there is an androgynous panicle (fig. 9, 20), with usually four to six opposite staminate catkins, the inflorescence terminated by a fifteen- to twenty-flowered pistillate catkin. *E. mexicana*, closely related to *E. pterocarpa*, probably has similar inflorescences, though only fruiting catkins are known. In *E. spicata* with its varie-

ties *Colebrookiana* and *aceriflora* the androgynous panicle (fig. 25, 43A) with its many-flowered pistillate catkin and its two to five staminate catkins, is often reduced to a three- to six-catkin staminate panicle (fig. 19, 24, 25, 43B) or to a solitary pistillate catkin (fig. 24, 43B). Two or three of these types of inflorescences may occur on the same tree. In the remaining species of the genus (*E. nudiflora* and its relatives) the staminate and pistillate inflorescences are always separate, and the plants are reported as dioecious. All herbarium specimens seen by the writer uphold this view, but further field studies should be made. The number of staminate catkins in the flower clusters of these species varies normally from five or four (*E. nudiflora*, fig. 22, 28) to three (*E. parvifolia*, fig. 23). Solitary staminate catkins are reported for *Engelhardtia* (see illustration of *E. nudiflora* in Hooker (1888)), but these reports are due mostly to inaccurate observations, for (1) the solitary catkin may be the remains of a panicle from which most of the catkins have dropped off, or (2) the common rachis of a panicle may be much shortened, and the catkins seem independent in a large or small group (*E. nudiflora*).



Fig. 12-23. Inflorescence diagrams; staminate catkins represented by light single lines, pistillate ones by heavy lines; new wood, new growth, by narrow twigs, old or mature wood by heavy twigs; leaves (compound) by half crescents.—Fig. 12-17. Deviation in the type and location of the inflorescences of *Engelhardtia chrysolepis*.—Fig. 18-23. Comparative positions of the inflorescences in *Engelhardtia*.—Fig. 14. Large compound panicle (from Petelot, no. 3031, flowers immature).—Fig. 15, 16. Small compound panicles, a staminate panicle above an androgynous one; the latter complete or the staminate catkins fallen off at x (fig. 16 from Lau no. 220).—Fig. 17. Fruiting stage of eight panicles, originally part of one (or two?) compound panicles.—Fig. 18. *E. chrysolepis*, panicles terminal on new growth.—Fig. 19. *E. spicata*, panicles in the axils of leaf scars on the old wood.—Fig. 20. *E. pterocarpa* and fig. 21, *E. nudiflora*, panicles in the axils of mature leaves on the upper part of the mature wood.—Fig. 22. *E. nudiflora*, panicles in the axils of mature leaves and of leaf scars lower down.—Fig. 23. *E. parvifolia*, panicles in the axils of leaf scars on the lower part of the mature wood.

In all the species of *Engelhardtia* except *E. chrysolepis* the inflorescences are lateral and subsessile on the old wood. In *E. spicata* and its varieties they occur in the axils of leaf scars, on the upper part of the old wood, developing usually about the time that new growth starts above the flowering portion of the twig (fig. 19, 24, 43). Eichler (1878) and Engler (1889) illustrate the pistillate catkins of this species in the axils of leaves on the new growth; the writer has seen no herbarium specimens in this condition, and the illustration may be that of another species. The inflorescences of *E. pterocarpa* (fig. 9, 20), *E. nudiflora* (fig. 21, 28, 44B), and *E. subsimplicifolia* (fig. 26, cf. fig. 44B) occur in the axils of leaves near the tip of the stem, seemingly on new growth, but the leaves are old, and the inflorescences are actually on mature wood just as in *E. spicata*. That these inflorescences are on old, not new, growth, although

they occur in the axils of leaves, is indicated by the condition shown in figure 27: inflorescence buds occur in the axils of mature leaves, probably at the end of the growing season. The catkins do not elongate until the dry season following this growing season. That the position is identical with that in *E. spicata* is shown by a few herbarium specimens of *E. spicata* var. *Colebrookiana* with similar undeveloped catkins axillary to leaves. In *E. spicata* and its varieties the leaves fall at the end of the growing season, and the catkins do not elongate until the new vegetative growth starts at the beginning of the following growing season. In *E. nudiflora* the leaves usually persist until the next growing season, and the catkins in the axils of these old leaves elongate before the new vegetative growth starts. The inflorescences of *E. parvifolia* (fig. 23) usually appear on the lower part of the old wood on leafless areas below a region which



Fig. 24-29. Flowering twigs, $\times \frac{1}{4}$.—Fig. 24. *Engelhardtia spicata* (from Bis Ram no. 99).—Fig. 25. *E. spicata* (modified after Blume, Flora Javae).—Fig. 26. *E. subsimplicifolia*.—Fig. 27, 28. *E. nudiflora*; fig. 27, staminate inflorescence buds in the axils of mature leaves and of leaf scars; fig. 28, flowering staminate panicles in the axils of leaves.—Fig. 29. *Carya ovata*; A, the bud scales fallen off; B, portion of another twig, the bud scales present.

has mature leaves but no flowers. An intermediate condition between this position and the usual position in *E. nudiflora* is occasionally present in the latter species—i.e., inflorescences occur in the axils of leaves on the upper part of the old wood and also on the leafless area just below this (fig. 22, 27, 44A), where the earlier formed leaves of the season's growth have fallen off because of hereditary or seasonal factors. Further observations in the field are needed to prove that the inflorescences in these species are always on old wood. In tropical plants such as *Engelhardtia* there is probably no sharp line of demarcation between one season's growth and that of the previous season, and there may be more than one growing season in one calendar year. Consequently it is difficult to tell whether an inflorescence occurs on the new season's growth, the new wood, or on the old and mature wood. Nevertheless this separation into old and new wood is of value, so far as it can be made, for the sake of description and comparison; this is especially true as the distinction is clearer in the temperate genera, and one should try to correlate the positions in the different genera.

In summary: The inflorescences of *Engelhardtia* may be panicles combining pistillate and staminate

catkins, or separate staminate panicles, or a separate pistillate catkin. In some species the plants are dioecious. There are two normal locations, terminal on the new growth or axillary on mature wood; in the latter case the inflorescences may appear in the axils of persistent leaves at the end of the growing season or in the axils of the leaf scars after the new growth begins above the flowering region. Figures 18-23 show the comparative positions of the inflorescences of the different species of the genus (see also fig. 41-44). In each species the staminate and pistillate inflorescences have identical positions—i.e., both are terminal or both are axillary.

Alfaroa (1 species, *A. costaricensis*).—The inflorescence of *Alfaroa* (fig. 10, 40) seems to be a solitary pistillate spike, but is really an androgynous panicle as in *Platycarya* and *Engelhardtia* spp. The central axis is pistillate with thirty to fifty flowers, and the side branches, usually only two in number at the base of the pistillate catkin, are staminate. However, these branches are extremely short, each consisting of only three to four flowers in a cluster, or even of a single flower. If the flowers are wind pollinated, as seems probable, the number of staminate flowers is very small, and it is possible that individual branches

or individual trees may produce longer staminate catkins. Such catkins have not as yet been found. The panicles are terminal on the main branches and on long leafy side branches.

Pterocarya (8 or 9 species).—In all species of *Pterocarya* the staminate and pistillate catkins occur in separate inflorescences. The pistillate catkins (fig. 30-32, 46, 47, 49) are always solitary, terminal, early-drooping, and, except for *P. Paliurus*, many-flowered. DeCandolle (1862) described the position as axillary, but the error was corrected by Oersted (1870) and by Eichler (1878). Eichler's figure, copied by Engler, shows the catkin as erect instead of drooping.

The type and position of the staminate inflorescences of *P. fraxinifolia* and of *P. stenoptera* have been considered by Eichler, Engler, and others as characteristic of the genus. Rehder and Wilson (1917) first described clearly the type and position of the staminate inflorescences in the different species of the genus. They divided the genus into two sections on the basis of bud structure and of the position of the staminate catkins.

Three different types of staminate inflorescences are now known. In *P. Paliurus* the catkins are in a two- to five-catkin panicle (fig. 30, 46), as in *Engelhardtia*. In *P. stenoptera* (fig. 31, 47), *P. fraxinifolia*, and other species of the section *Eupterocarya* exclusive of *P. Paliurus*, the catkins are solitary, but on a short, thick stalk, which bears at the base of the catkin two to four small deciduous bracts, leaving definite scars. This stalk (s, fig. 31) is formed the year previous to the opening of the flowers and consequently is thicker than the axis of the catkin. The bracts of *P. stenoptera* and its relatives correspond to the bracts at the base of the individual catkins of the cluster of *P. Paliurus*. In all these species the winter buds (fig. 31A) are naked, not scaly, and the inflorescences occur near the tip of the old wood, in the axils of scars of last year's leaves, as in *Engelhardtia spicata*; rarely, one catkin occurs in the axil of the lowest leaf of the new growth (fig. 31B; compare Oersted (1870)).

In *P. rhoifolia* and its relatives of the section *Chlaenopterocarya*, the winter buds (fig. 32, A, B) have two broad valvate bud scales which are early deciduous during the winter. The staminate catkins are solitary and occur in the axils of the bud scales (fig. 32C, 49), hence at the base of the new growth. An additional catkin may occur in the axil of the lowest foliage leaf. The flowering portion of the twig is very much condensed. These bud scales may be the modified leaves at the tip of the old growth, corresponding to those subtending the catkins in *P. stenoptera*, so that the condition in *P. rhoifolia* is not very different from that in the latter species. In *P. rhoifolia* the stalk of the catkin is very short, slender, and not clearly visible, but has one or two hairy bracts (b, fig. 32C) corresponding to those in *P. stenoptera* and *P. Paliurus*.

Juglans (15 species).—The inflorescences are nearly uniform throughout the genus, and the type and

arrangement are well known. The solitary pistillate catkin is terminal on the new growth (fig. 11A, 48) as in *Pterocarya*, but is only partially drooping or actually erect at flowering time, and has fewer, less crowded flowers. *J. Sieboldiana* (fig. 11C) has the largest number of flowers (12-20), approaching the condition in *Pterocarya stenoptera* and exceeding the number in *P. Paliurus*, while *J. cinerea* and related Chinese species may have nearly as many. *J. nigra* (fig. 11B), *J. regia*, and their relatives may have only three, two, or one flower in the cluster. As in *Pterocarya* the peduncle of the catkin may have one or two bracts, often with buds in their axils, these corresponding to the bracts subtending staminate catkins in the androgynous panicles of *Platycarya*, *Engelhardtia*, and *Alfaroa*.

The staminate catkins are densely flowered, solitary, though often superposed, sessile, and axillary on the upper part of the old wood (fig. 11A, 48), occurring as in *Pterocarya stenoptera* above the leaf scars of the fallen leaves of the previous season. This is the only case in the family where the catkins are both solitary and sessile. Although a peduncle is not developed, there are two to four bud-scale-like bracts at the base of the catkin, corresponding to those on the peduncle of the *Pterocarya* staminate catkins. These bracts are very inconspicuous, but are visible during the winter as well as when the catkins elongate in the spring (b, fig. 11A).

Carya (18 or 19 species).—There is very little variation in the type and location of the inflorescences. The pistillate catkin (fig. 29A, 33A, 50, 51) is solitary, terminal, mostly erect, and few-flowered, the number of flowers varying from ten to one. *Carya pecan* has the largest number of flowers. The bracts of the peduncle are usually present, the buds in their axils often well developed.

The staminate catkins (fig. 29, 33, 50, 51) are normally in a cluster of three, at the end of a short or rather long common peduncle, as in *Engelhardtia parvifolia*, etc. The bracts subtending the two lateral catkins of the cluster are well developed, and usually persistent and conspicuous. A bract is sometimes united for a short distance with the stalk of the individual lateral catkin. Very rarely the number of catkins may be reduced to two or one (*C. floridana*), but these cases are abnormalities.

The location of the staminate inflorescences is fundamentally uniform in the genus, at the base of the new wood, in the axils of bud scales, as in *Pterocarya rhoifolia*; in *Carya*, however, the number of bud scales and of catkin-clusters is much larger. In most species, as in *C. ovata* (fig. 29, 50), the inflorescences occur at the base of the new growth of the main terminal twigs, which bear the pistillate flowers at their tips, and may also appear at the base of well developed axillary branches arising from last season's growth. In *C. pecan* and its relatives a somewhat different condition occurs. For example, in *C. cordiformis* (fig. 33A, 51) the staminate inflorescences appear not only on the main twigs as in *C. ovata*, but



Fig. 30-33. Flowering twigs; fig. 30, $\times \frac{1}{2}$; others, $\times \frac{1}{4}$.—Fig. 30. *Pterocarya Paliurus*, staminate flowers unopened.—Fig. 31. A, *P. fraxinifolia*, tip of twig in winter; B, *P. stenoptera*, twig in flower; one of catkins in the axil of a leaf; thickened stalk *s* and bracts *b* at base of staminate catkins; *b'* bract scar.—Fig. 32. *P. rhoifolia*, A, B, tips of twigs in winter, the bud scales present and fallen off; C, twig in flower, unusual condition where the bud scales have persisted until flowering time; *b*, inflorescence bract.—Fig. 33. A, *Carya cordiformis*; B, C, outline sketches; B, *C. floridana*, portion of this season's growth, staminate panicles in the axils of bud scales and leaves (modified after Sargent); C, *C. pecan*, part of old wood, staminate panicles in the axils of bud scales of short side branch.

also on short side branches from the old growth. Furthermore, these branches become progressively shorter down the twig, the leaves being reduced to two or one or even absent, and the inflorescences reduced in number to two, in the axils of the principal scales of the axillary bud, with the terminal bud of the branch between them. In this last case the terminal bud of this special branch does not grow, and it often falls off early. These branches, then, are special flowering branches. In *C. pecan*, itself (fig. 33C), the common peduncle of the cluster is very short, catkins are commonly produced only on the special flowering branches on the old wood, and the general appearance of a flowering twig is very different from that of *C. ovata*. The naked catkins appear to be on the old wood, resembling the condition in *Juglans*. But the series of transitional conditions described above for *C. cordiformis* and *C. pecan* show that these inflorescences are definitely on the new wood, just as are those at the base of the main shoots. There is a discussion of this condition of *C. pecan* by Sargent (1895) and by Trelease (1896),

with the same conclusion, but the drawings and discussions do not bring this out clearly. Gray's Manual (1908) separates *C. pecan* from the other species in the northeastern states on the location and appearance of the staminate inflorescence, but this is not a safe distinction, as the length of the common peduncle varies considerably and there is intergradation with the condition in *C. cordiformis*, leading toward *C. ovata*. Trelease and Sargent use the position of the staminate inflorescences as one of the characteristics of the section *Apocarya*, but as shown by the writer, it can not be used as an absolute character; furthermore, *C. myristicaeformis*, a member of this section, has the location of the catkins as in *C. ovata*; the bud scale condition in that species is also transitional to that in the section *Eucarya*.

Sargent (1913, 1933) describes the staminate inflorescences of *C. floridana* as in the axils of foliage leaves of the new growth (fig. 33B). A few herbarium specimens do show this condition, but other herbarium specimens and field studies by the writer indicate that in this species the catkins occur nor-

mally in the axils of bud scales, and the condition cited by Sargent is abnormal.

Clearly, then, the staminate inflorescences in *Carya* occur in the axils of bud scales, on the new growth, a position different from that in *Juglans*, but similar to that of *Pterocarya rhoifolia*.

DISCUSSION.—The type of inflorescence.—In the *Juglandaceae* there are four principal types of inflorescences (fig. 18–23, 39–51)—viz., (1) a cluster of several staminate catkins combined with a central catkin which is largely or entirely pistillate, (2) a solitary pistillate catkin, (3) a cluster of staminate catkins ranging from six to two, and (4) a solitary staminate catkin.

These inflorescences are fundamentally similar, and there are intermediate types between them in various species. It is evident, therefore, that the members of the family are closely related and have been derived from a common ancestral stock. The inflorescence of this ancestral stock was undoubtedly a panicle. This is indicated by the fact that the inflorescences of the primitive genera of the family are panicles, and all other types of inflorescences in the family are easily derived from these panicles by reduction—that is, merely by an abortion of one or more branches, or catkins. The different stages in reduction through which the inflorescences must have passed in evolution from the primitive-complex to the advanced-simple types of inflorescences are represented in various species of the modern genera. That there has been a reduction series is indicated by the following: (1) there are both primitive and advanced types of inflorescences in some species, usually on the same tree; (2) in *Alfaroa* the lower branches, catkins, of the panicle are reduced to one to three flowers, in more advanced genera these catkins are entirely aborted; (3) in the more reduced types of inflorescences the position of aborted branches is usually indicated by the presence of bracts at the proper places.

Figures 39–51 show the inflorescences of the different genera arranged from primitive to advanced types. The androgynous cluster or panicle (represented in *Platycarya*, *Alfaroa*, *Engelhardtia* spp., fig. 39A, 40, 41A, 43A) is the first or most primitive type found in modern genera. The abortion of the pistillate catkin would result in a cluster of staminate catkins (*Platycarya*, *Engelhardtia*, *Pterocarya Palurus*, fig. 39B, 43B, 46). The abortion of the staminate catkins of an androgynous panicle would result in the solitary pistillate catkin (*Engelhardtia*, *Pterocarya*, etc., fig. 41B, 43B, 44B, 46, 47, 49). The evolution in the pistillate inflorescence would be from such a long, many-flowered catkin through a shorter, fewer-flowered, more or less erect catkin or spike (*Juglans*, fig. 48) to a very short, finally one- or two-flowered spike (*Carya*, fig. 50). In the staminate inflorescences the abortion of branches would change a five- or six-catkin panicle into a three-catkin inflorescence (*Engelhardtia*, *Carya*, fig. 23, 44, 50, 51). From this inflorescence the solitary catkin is easily

derived. In *Pterocarya stenoptera* the stalk of the solitary catkin (s, fig. 31, 47) shows very clearly the bracts or their scars indicating the position of aborted catkins. The solitary sessile catkin, found in *Juglans* (fig. 48) is the most advanced type; here the bracts are present also, but very small and inconspicuous because of the absence of a stalk.

Parkin (1914), as well as the writer, considers the panicle more primitive than the catkin, the latter type having come from the former by reduction. But he believes that the solitary catkin has been derived from the panicle by the "loss of the tertiary axes." The writer believes that the solitary catkin in the *Juglandaceae* was derived from the panicle by complete abortion of the side rays of the panicle—that is, the lower and larger secondary axes with their tertiary axes.

From the discussion above, it is evident that the typical inflorescence in the *Juglandaceae* is not the catkin, as usually stated, but a panicle, of which the catkin is only the branch or ray. This cluster of catkins, the panicle, of the primitive members of the *Juglandaceae* differs in several respects from the characteristic highly branched panicle such, for example, as that of *Rhus*. Thus the main rachis is often much shortened, and the rays, or catkins, are deciduous, usually drooping, and not secondarily branched. The modern *Juglandaceus* panicle is, therefore, a somewhat specialized one—that is, modified from the probably typical panicle of the ancestral *Juglandaceus* stock.

Some remnants of the characteristic features of the panicle are to be found in certain species. Thus, (1) the panicle in *Platycarya* is erect; (2) the main rachis is rather elongated in the androgynous inflorescences of *Engelhardtia spicata*, *E. pterocarpa*, and some inflorescences of *Platycarya*; (3) a type of secondary branching of the rays of the panicle occurs in certain inflorescences of *Engelhardtia chrysolepis* and of *Platycarya*—that is, the inflorescence is a "compound panicle," consisting of several clusters of catkins combined in one large inflorescence at the tip of the stem (fig. 7, 14, 15, 36). This compound panicle resembles the usual highly branching panicle. The individual catkins of the lower clusters of this compound panicle correspond almost exactly to the side branches of the ray of an ordinary panicle. The "simple" panicle—a single cluster of catkins—normally present in the modern *Juglandaceae* is thus the uppermost part of the ancestral panicle, the lower part having disappeared in the process of evolution. It is significant that the upper rays of an ordinary panicle are branched only slightly secondarily and are similar therefore to the catkins with their flowers in a single series.

There is some indication that each individual catkin may have been derived from a branched ray of a panicle. For example, the flowers of the catkins of *Engelhardtia chrysolepis* (fig. 8) and of *E. pterocarpa* (fig. 9) are loosely arranged and are in groups along the catkin, suggesting the reduction of branches.



A complete parallel evolutionary series from a complex branching panicle to a simple cluster of unbranched catkins may be seen in the modern members of the genus *Rhus* of the *Anacardiaceae*. In well developed staminate panicles of *Rhus copallina* the panicle is very diffuse, each ray branching two or three times (fig. 34A). In the more poorly developed pistillate panicles (fig. 34B) there is much less branching, the tips and secondary branches of the rays usually being simple. *Rhus canadensis* shows what happens if this simplification continues. Very strongly developed inflorescences of that species (fig. 35) show a long naked rachis with several nearly sessile spikes on the upper portion and stalked clusters of spikes along the lower part. This type of inflorescence corresponds exactly to the compound panicle of *Engelhardtia chrysolepis* (fig. 14, 15, 36, clusters of catkins, a-e, correspond to those of fig. 35; see discussion in preceding paragraphs). Some individuals of *Rhus canadensis* have a smaller cluster of spikes or catkins (fig. 37), very closely resembling the simple panicles of the primitive genera of the *Juglandaceae* (fig. 38). The single series of flowers along the spikes, each flower subtended by a bract and two bracteoles, is identical with the arrangement of the flowers in the catkin in the *Juglandaceae*. This reduction series in *Rhus* shows how easily the type of panicle found in the *Juglandaceae* may have evolved.

The position of the inflorescence.—There are three principal places on the twigs where the inflorescences may be located (fig. 18-23, 39-51): (1) at the tip of the main stem or principal branches, thus terminal on the new growth, (2) lateral in the upper part of the old growth, in the axils of leaf scars or persistent leaves, (3) lateral at the base of the new growth, in the axils of bud scales.

The terminal inflorescences are usually pistillate, or they combine pistillate and staminate catkins. The latter condition occurs in *Platycarya*, *Alfaroa*, and *Engelhardtia chrysolepis* (fig. 18, 39A, 40, 41A). The independent terminal pistillate catkin occurs as a variation in *E. chrysolepis* (fig. 12, 41B), and as a characteristic feature in *Pterocarya*, *Juglans*, and *Carya* (fig. 46-51). Only in *Platycarya* and *Engelhardtia chrysolepis* are the independent staminate panicles terminal (fig. 13, 39B), and then only on individual branches or trees.

Fig. 34-51.—Fig. 34-38. Series of diagrams to show possible origin of a panicle such as occurs in the primitive members of the *Juglandaceae*. Each single line represents a flower bearing axis, the half crescents compound leaves.—Fig. 34. *Rhus copallina*. A, one side of a highly branched lower ray of a staminate "compound" panicle; B, one side of a less branched pistillate "compound" panicle.—Fig. 35. *Rhus canadensis*, a similar "compound" panicle, made up of catkins, these grouped into five simple panicles (a to e).—Fig. 36. *Engelhardtia chrysolepis*, similar "compound" panicle, made up of six simple panicles of catkins (a to e), the common rachises of the panicles foreshortened.—Fig. 37. *Rhus canadensis*.—Fig. 38. *E. chrysolepis* or *Platycarya strobilacea*. Simple panicles (a) composed of catkins.

Fig. 39-51. Series of diagrams to show evolution in type and position of the inflorescence in the *Juglandaceae*, from primitive to advanced genera. For symbols see fig. 12-23. Only persistent leaves on old wood shown.—Fig. 39. *Platycarya strobilacea*.—Fig. 40. *Alfaroa costaricensis*.—Fig. 41. *Engelhardtia chrysolepis*.—Fig. 42. Theoretical ancestor of other species of *Engelhardtia*, no inflorescences terminal on main shoots.—Fig. 43. A, B. *E. spicata*.—Fig. 44. *E. nudiflora*; A, staminate tree; B, pistillate tree.—Fig. 45. Ancestral genus, with primitive type and position of inflorescences, from which the genera with inflorescences in two different positions must have originated.—Fig. 46. *Pterocarya Palturus*.—Fig. 47. *Pter. stenoptera*.—Fig. 48. *Juglans cinerea*.—Fig. 49. *Pter. rhoifolia*.—Fig. 50. *Carya ovata*.—Fig. 51. *C. cordiformis*.

Axillary inflorescences are mostly staminate. Only in species of *Engelhardtia* are the pistillate catkins, independent or in combination with staminate ones, axillary (fig. 20, 43A, 43B, 44B).

Inflorescences which occur in the axils of foliage leaves are mostly on the old wood. This location is clear because the leaves have fallen at flowering time, and the new growth is at least partly developed above the flowering area (*E. spicata*, *Pter. stenoptera*, *P. Palturus*, *Juglans* spp. fig. 19, 43, 46-48). Two apparent exceptions occur. (1) In *Engelhardtia chrysolepis* and *Platycarya* the inflorescences that occur occasionally at the tips of very short side branches are on the new growth (fig. 2, 6, 18, 41A); but although these inflorescences appear axillary, they are terminal on the short branches. This position is not characteristic of these species, as distinct terminal ones are present also. (2) The inflorescences of *E. pterocarya* and of *E. nudiflora* and its relatives (fig. 20-22, 44) occur mostly in the axils of leaves, not leaf scars, and thus appear to be on the new growth, but, as explained in the description of that species, this flowering region may be interpreted as mature wood. In tropical trees, however, the distinction between old and new wood is not clear cut, and the exact year's growth is somewhat uncertain.

Only the staminate inflorescences of *Pterocarya rhoifolia* and its relatives, and of the species of *Carya*, occur in the axils of bud scales (fig. 49-51). The origin of this position is discussed later.

There is a definite evolutionary sequence of the positions of the inflorescences in the family. The writer believes that the inflorescence of the ancestral stock of the *Juglandaceae* was terminal in position because (1) many inflorescences in the family are terminal, (2) it is easy to derive all other locations of the inflorescences in the family from such a position, and (3) this is the position of both kinds of inflorescences in species of the modern primitive genera of the family. Their primitive nature is indicated by the evolutionary trends of the type of inflorescence in the family as discussed in the preceding pages and by uncompleted studies by the writer on the structure of the flowers.

Figures 39-51 show the inflorescences of the members of the *Juglandaceae* arranged from primitive positions to advanced ones. The pistillate and stami-

nate inflorescences have evolved for the most part along independent lines from a common origin.

The primitive terminal position persists in most of the inflorescences which contain pistillate catkins, as discussed in the paragraphs above. Another line of evolution, mostly for the staminate flower clusters, was toward an axillary position, in the axils of foliage leaves, fallen or present, on the old wood, and finally to the axillary position in the axils of bud scales, on the new wood. The steps leading up to these last two positions are easy to follow. As shown in *Platycarya* and *Engelhardtia chrysolepis*, terminal inflorescences of the primitive members of the family would have occurred at the tips of short or long lateral branches as well as at the tips of the main axes. Since the axillary branches in the upper part of last season's growth would also bear terminal inflorescences (fig. 41, 42, 45), it is easy to derive the short-stalked axillary inflorescences in this location by the reduction in length of the branch and a loss of leaves accompanying this. Such a reduction in branches is shown clearly in *Platycarya* and in *Engelhardtia chrysolepis*, though in these species on the new growth.

Undoubtedly the location of flower clusters in the axils of bud scales is a specialized condition. Bud scales do not occur in the tropical primitive members of the family (*Engelhardtia*, *Alfaroa*, and *Pterocarya*, section *Eupterocarya*); scaly buds are certainly more specialized than naked buds and are associated with specialization for colder or drier climates. The staminate catkins of *Carya*, and also of *Pterocarya rhoifolia*, are usually described as axillary at the base of new growth. There are two possible explanations of this location. The position on the new growth could have evolved from a reduction and then localization of branch inflorescences on the new wood such as those which occur occasionally in *Platycarya* and in *Engelhardtia chrysolepis*. The abnormal inflorescences of *Carya floridana* (fig. 33B) indicate such a possible interpretation. However, another interpretation is more probable. No members of the *Juglandaceae* normally have inflorescences on the new wood, and *Carya* would therefore be different in this respect from the other genera. This discrepancy may be explained by studying the position of staminate catkins in related species of the genus *Pterocarya* (fig. 30-32, 46, 47, 49). *P. stenoptera* and *P. palurus*, which have naked buds, have their catkins on the old wood in the axils of the upper leaves, which have usually fallen off by flowering time. In *P. rhoifolia* bud scales are present, and the staminate catkins appear only in their axils. These bud scales may be interpreted as the uppermost last season's leaves, telescoped into smaller space by the reduction of internodes, and modified into protective coverings for the bud. Thus the location of these catkins is only a specialized condition of the lateral position on the old wood. This may, however, represent a different line of evolution from a tropical ancestor lacking clear distinction between old and new wood.

The inflorescence of the pre-Juglandaceae.—It is evident from the discussion of the last few pages that

the inflorescence of the original stock from which the *Juglandaceae* sprung was a terminal highly branched panicle.

The individual flowers of this panicle were perfect. That this is true is indicated by the combination of pistillate and staminate catkins in one panicle in three genera, by the transition of pistillate to perfect to staminate flowers in the same catkin (*Platycarya*), and by the presence in many species of an abortive pistil in the staminate flowers (to be discussed in a later paper).

It is suggestive that the inflorescences in the *Anacardiaceae* fulfill all these ancestral requirements. Furthermore there are many other features in common between the two families, such as pinnately compound leaves, abundance of resin, absence of stipules, tendency toward imperfect flowers, a solitary ovule, etc. Without a more thorough knowledge of the comparative flower structure in the *Anacardiaceae*, however, it is impossible at present to be positive of a close affinity between these families. Other families, too, have certain features in common with the *Juglandaceae*—for instance, the *Julianiaceae* with its compound leaves and paniculate inflorescences, and the *Betulaceae* with similar clusters of catkins.

The evolutionary position of the genera in the Juglandaceae.—The "simple" terminal androgynous panicle, a single cluster of unbranched rays, catkins, one of these pistillate, the others staminate, is the nearest approach in the modern *Juglandaceae* to the ancestral highly branched panicle of perfect flowers.

That type of inflorescence occurs in *Platycarya*, *Alfaroa* (fig. 30A, 40), and in one species of *Engelhardtia* (fig. 41A). Judged by their inflorescences, these genera may be considered primitive genera. Within the genus *Engelhardtia* there is a great deal of variation or evolution along independent lines. *E. chrysolepis*, with its terminal androgynous panicle, is the most primitive species; *E. spicata*, *E. pterocarya*, and probably *E. mexicana*, with their axillary androgynous panicles (fig. 20, 43) are not much more advanced. In both of the first two species the androgynous panicle is often reduced by abortion to either a staminate cluster or to a solitary pistillate catkin (fig. 43B). The remaining species of the genus are more specialized than those mentioned above, as the sexes are always separate and the trees are dioecious (fig. 44). Minor variations in the location of the inflorescence in these species were discussed under that genus. It is interesting that nearly all the features of inflorescence present in the more advanced genera are found in different species of *Engelhardtia*, and this genus is probably very close to the ancestor of all the genera of the family.

In each species of the three genera mentioned above the staminate and pistillate inflorescences have identical positions—that is, both are terminal or both are axillary (fig. 39-44). In the species of the remaining genera the pistillate and staminate catkins have different positions on the plant, the pistillate terminal, the staminate axillary (fig. 46-51). These genera are therefore more advanced. They must have evolved

from an ancestor (fig. 45) which had androgynous inflorescences terminal on the new growth and terminal on branches from the old wood, from which latter position a subsessile axillary position is easily derived. Such an ancestral condition occurs in *Engelhardtia chrysoplepis* (fig. 41A), *Platycarya*, and *Alfaroa*. A parallel evolution for the staminate inflorescences is shown by the advanced species of *Engelhardtia* which have axillary staminate panicles similar to those of *Pterocarya Paliurus* and of *Carya*.

In all species of *Pterocarya* (fig. 46, 47, 49) the pistillate inflorescence is a solitary terminal elongated catkin resembling that of the reduced form of *Engelhardtia chrysoplepis*. Through *Pterocarya Paliurus*, which has two to several staminate catkins in an axillary panicle on the old wood, there is a connecting link between the staminate inflorescences of the genus *Engelhardtia* and the other species of *Pterocarya*, which have solitary catkins. As explained earlier, *P. rhoifolia* and its relatives have evolved along a different line, having their staminate flower clusters in the axils of bud scales.

In its inflorescences *Juglans* (fig. 48) is very closely related to *Pterocarya*, as the pistillate catkin is terminal, and the staminate is solitary and axillary on the old wood. But the inflorescences are more advanced in that the pistillate is more or less erect and is fewer flowered, and the staminate catkin is sessile with only slight indication of its reduction from a panicle. In the staminate inflorescence *Juglans* is more advanced than any other genus of the family.

The solitary terminal pistillate catkin of *Carya* (fig. 50, 51) is closely similar to that of *Pterocarya* and *Juglans*, but is further reduced. The staminate inflorescence is quite different in type and position from that of *Juglans*. The peduncled cluster of three catkins resembles the staminate inflorescence of *Engelhardtia parvifolia* and is easily derived from such a staminate panicle as that of *Pterocarya Paliurus*. In its location in the axils of bud scales the inflorescence of *Carya* resembles that of *Pterocarya rhoifolia* (fig. 49), but that species has solitary catkins. It is highly probable that *P. rhoifolia* and *Carya* spp. had a common ancestor and have evolved along parallel lines. The inflorescences of the *Carya cordiformis* and *C. pecan* types usually occur on special flowering

branches from the old wood (fig. 51). As a whole *Carya* may be considered the most advanced genus in the family in its inflorescences, although in its type of staminate inflorescence the genus is not so greatly advanced as *Juglans*.

SUMMARY

The inflorescences of all six genera of the family are described and figured.

A possible evolutionary series from primitive to advanced types of inflorescences is proposed, and the probable type of inflorescence of the ancestors of the family is described.

The inflorescence of the pre-Juglandaceae stock was probably a terminal highly branched panicle of perfect flowers.

In the modern *Juglandaceae* the inflorescence of primitive members is a terminal androgynous panicle, combining one central pistillate and several staminate branches or catkins. From this condition there is an advance to inflorescences entirely pistillate or entirely staminate. In the pistillate flower clusters the solitary catkin remains terminal, or rarely becomes axillary, and in the most advanced genera is reduced to a few-flowered spike. In the staminate inflorescences the advance is from a terminal position to an axillary one, first in the axils of foliage leaves on the old wood, then in the axils of bud scales, and from a several-catkin panicle to few-catkin clusters to a solitary, finally sessile catkin.

In the *Juglandaceae* each catkin represents one branch of a panicle, and the solitary catkin is the most advanced type of inflorescence, the branches of the panicle having disappeared through abortion.

Engelhardtia, *Alfaroa*, and *Platycarya* have primitive types and positions of inflorescences, though there is considerable advance in some species of *Engelhardtia*. *Pterocarya* has advanced types, while the inflorescences of *Juglans*, similar to those of *Pterocarya*, are still more advanced. The type of staminate inflorescence of *Carya* is rather primitive, but the location is a specialized one; the pistillate flower cluster is the most advanced in the family.

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MORPHOGENETICAL STUDIES IN THE DEVELOPMENT OF SUCCESSIVE LEAVES IN ASTER, WITH RESPECT TO RELATIVE GROWTH, CELLULAR DIFFERENTIATION, AND AUXIN RELATIONSHIPS¹

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It is the purpose of this paper to report an investigation of the development of both the rosette and cauline leaves in two inbred species of *Aster* and in reciprocal hybrids resulting from a cross between these species. Specifically an attempt was made to study the morphological processes by which the ultimate form of the leaf is attained in these plants, particular emphasis being placed on cell numbers, orderly sequence of cell division, cellular differentiation, and relative growth rates in the leaf as a whole and in various regions of the leaf. Along with these morphogenetical studies, the auxin output of these leaves, as measured by auxin diffusions, has been determined and its distribution in relation to the pattern of growth in the leaves noted. An analysis of this pattern of growth is attempted in order to determine whether a differential regional distribution of auxin within the leaf might be correlated with differential growth of the leaf. Finally there is reported a study of the developmental pattern for the successive leaves in these plants. The question arises as to whether or not the relationships of length and breadth of leaf are constant throughout the entire life of the plant. This investigation points out that such relationships do change and indicates when and where they occur. An explanation of the biological factors underlying such changes, however, is not at all clear.

MATERIALS AND METHODS.—Two species of *Aster*, *A. novae-angliae* L., *A. multiflorus* Ait.² and their reciprocal hybrids were used in these investigations. The developmental studies were based on the cotyledons and the first twenty leaves of the seedlings. These were chosen because they afforded the opportunity of studying the relative growth rates of the rosette leaves and of those cauline leaves appearing with and subsequent to the initiation of stem elongation. Leaf studies were also made on the second year's growth as well, again both from the rosette and the elongating axis.

The leaves of these plants were chosen because the species had been inbred for two or more generations over a period of three years, during which time the writer has attempted to familiarize himself with their genetical trends, their comparative morphology, and

with certain aspects of the correlation between their auxin activity and organization.

Both leaf measurements and cell measurements were made from the surface of the leaf. Leaves were chosen for investigation because they are numerous and accessible and all stages of their development are found on the same plant. The epidermal cells were chosen for cell measurements because they are the most accessible of the tissues of the leaves and because they can be studied in the living condition. Mounts (1932) has already pointed out the mechanical function of the epidermis and has indicated that it is the only compact tissue between veins which supports the interior mesophyll of the islets. The epidermis, the cells of which are entirely devoid of intercellular spaces, is a complete, compact envelope, covering the whole area of the leaf. It is very unlike the subjacent mesophyll whose cells in the mature leaf form a very loose spongy tissue provided with large intercellular spaces. For this reason, its development has been chosen to serve as an index of the growth of the leaf as a whole. However, as Avery (1933) has already pointed out, there must be interacting stresses and strains which are imparted to the epidermal cells in the development of the palisade and spongy mesophyll tissues. Therefore, the use of epidermal cell size as an index of foliar development lends itself to criticism. However, the following graphs indicate specific trends, despite the interacting distorting stresses and strains imparted by the spongy mesophyll. Inasmuch as these tend always to retard measurable growth, quite obviously the epidermis never has a full chance to express itself fully and reach complete undisturbed development. This can mean only that the resulting curves would be even more striking than actually. Kolkunov (1905) demonstrated the necessity for the selection not only of comparable leaves of the same age but also of corresponding parts of those leaves by showing that the anatomical coefficients³ of the leaves vary with their position on the stem. Yakushkina and Vavilov (1912) stressed the necessity for detailed comparative studies of one single part of the tissue of the leaf. Kolkunov (1905) also discovered in his studies on transpiration that cell size, or more precisely, the tendency under certain definite conditions to form cells of a definite size, is a strictly inherited character.

Because of the prominence of the midrib the epidermis overlying the midribs is made more conspic-

³ The size and number per unit area, of epidermal cells, stomata, guard cells, veins, and mesophyll cells, served as his anatomical coefficients.

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² Each of the two species has $n=5$ chromosomes.

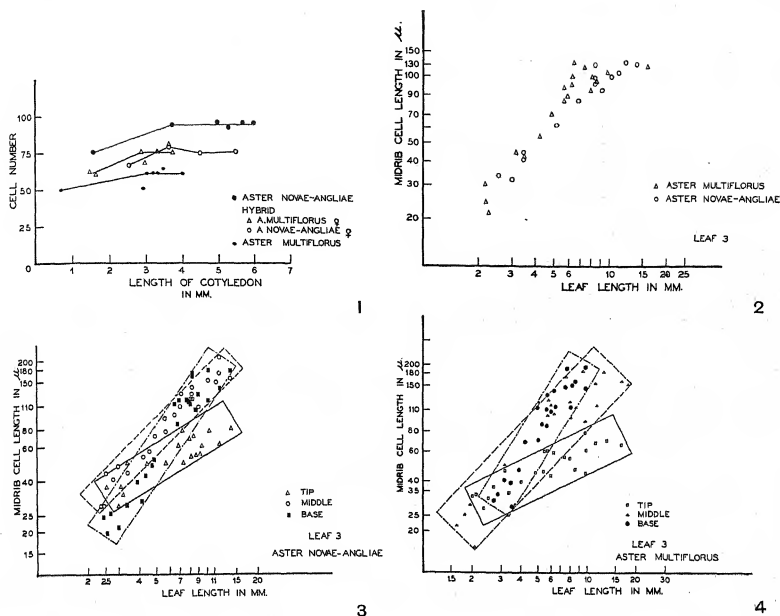


Fig. 1-4.—Fig. 1. Relationship between cell number and length of mature cotyledon in *A. novae-angliae*, *A. multiflorus*, and their reciprocal hybrids. Observations based on averages from 150+ plants.—Fig. 2. Relationship between cell length and leaf length. Variability of size of cells in older leaves due to irregularities in the cell wall.—Fig. 3. Relationship between cell size in three regions of the leaf and leaf size. In this and subsequent graphs, data plotted directly and enclosed in rectangles to show variability and to indicate trends of development. The third leaf in *A. novae-angliae*. Averages from 150-200 plants.—Fig. 4. Relationship between cell size in three regions of the third leaf of *A. multiflorus*.

uous than that elsewhere, and since this portion of the epidermis is the least obscured by epidermal hairs or glands, and especially since the cells of this region are oriented in the long axis of the leaf, it was chosen for length measurements. Results were at first tabulated for both the upper and lower epidermis and found to check well, so that it was felt that information based on studies of the lower epidermis would be representative for the upper and lower epidermis and therefore suggestive for the leaf as a whole.

The length of the cell was arbitrarily considered to be that dimension of the cell which was parallel to the midrib of the leaf and to the long axis of the entire leaf, and the width of the cell as that dimension corresponding to the width of the leaf. Some measurements were made on fixed material, though all were checked on living leaves, thus avoiding the

possibility of error by shrinkage or distortion due to fixation.⁴

In the study of development and shape, use was made of the compound interest formula (Huxley, 1932) which can be used to interpret the patterns of heterogonic or isogonic growth—i.e., the growth of an organ which is growing at a rate different from or at the same rate as the body.⁵

⁴ No distortion or shrinkage were found at any time. Measurements were made with an ocular micrometer and all measurements on each leaf represent the averages of at least 100 to 200 cells per leaf. This was expressed as the arithmetical mean, $\bar{m} = \Sigma m/n$. The standard deviation of the ungrouped data, $\sigma = (\Sigma d^2/n)^{1/2}$ and the probable error were also calculated from random samples of sets of measurements and found to be well within the range of statistical soundness.

⁵ In the formula $y = bx^x$, x representing length of a leaf, y its breadth, and b a constant denoting the value

TABLE 1. Relative length of mature cotyledon and first leaves in *Aster* hybrids.

	Length in mm.				
	<i>A. multiflorus</i> × <i>A. multiflorus</i>	<i>A. novae-angliae</i> ♀ × <i>A. multiflorus</i> ♂	<i>A. multiflorus</i> ♀ × <i>A. novae-angliae</i> (<i>albus</i>) ♂	<i>A. multiflorus</i> ♀ × <i>A. novae-angliae</i> ♂	<i>A. novae-angliae</i> × <i>A. novae-angliae</i>
Cotyledon	4.1	6.9	3.5	4.0	5.1
1st leaf	10.0	12.9	7.2	7.9	7.8
2nd leaf	11.2	14.5	7.8	9.2	8.5
3rd leaf	13.8	16.7	8.5	11.4	9.3
4th leaf	16.7	18.9	9.1	13.7	11.0
7th leaf	30.3	30.4	20.8	24.3	21.1

Average standard deviation for cotyledons ± 0.09 ; for rosette leaves ± 1.2 .

RESULTS.—*Cotyledons*.—A study of the development of the cotyledons excised from ungerminated

of y when x is 1, a straight line will result when these two dimensions x and y are plotted against each other logarithmically, provided the rate of change in relative size of x and y be constant. The slope of the resulting line, termed k or growth coefficient, measures the relative rates of growth in length and breadth of the leaf. If the width of leaf increases at the same rate as the length, the value of k will be 1, and the slope of the line, when plotted, will be 45° . If, however, the length should grow at a faster rate than the width, the slope of the line will be less than 45° (when length is represented on the x -axis) and the value of k will be less than 1. The ratio of length to breadth, or the shape index (Sinnott, 1927) is different in these two cases. It is also perfectly possible for k (or the slope of the line) to shift even in the development of a single organ as in *Capsicum* (Kaiser, 1935). The dimensions (length and breadth), for instance, of a young ovary may grow at the same rate and yield a curve whose slope is 45° . It is equally possible, as was shown by Sinnott and Kaiser (1934) in *Capsicum* for the same fruit to increase in length after fertilization at a considerably faster rate than in width due to a post-fertilization stimulus. The slope of the line will then be different from that which it was at first and thus there results a line whose slope has changed, suggesting a different shape index. Shape differences may therefore be due to differences in the value of k .

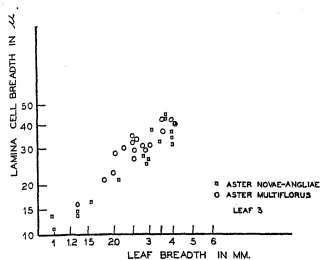
It is equally conceivable that if the meristem of a young organ is smaller at the outset than that of another homologous organ whose shape index is the same and whose dimensions of length and breadth develop at the same rate as the smaller one, the two lines resulting from a logarithmic plot of these values will not only be of similar slope, but an extension of either would give a continuation of the other. One line, therefore, will be nearer the origin than the other because its absolute length and absolute breadth at any given moment are lesser. This is a difference in the value of b in the formula used by Huxley and may be due to an initial difference in the relative sizes of the "anlagen."

Differences in shape types may be due to the differences in the "initial capital" or to the time at which genes concerned with shape become effective. In Cucurbits, so extensively studied by Sinnott, the differences in shape types are due not only to differences in the "initial capital" but to the fact that shape genes are operative in the earliest primordia. In *Capsicum* fruits, however, the stage of development at which shape genes produce their visible effect is a rather late one so that shape differences are due to differences in the value of k (to changes in the slope of the line). In the following analysis of growth pattern in *Aster* it will be seen that at least one important difference between rosette and stem leaves is due to a change in b in these leaves.

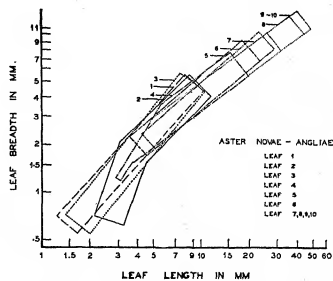
embryos removed from the achene has shown the number of epidermal cells along the future midrib to be fairly constant and characteristic of the species studied. Figure 1 indicates graphically the relationship between cell number and cotyledon length in the two parents and their reciprocal hybrids. It will be seen that the two hybrids have approximately the same number of epidermal cells in the linear extension of the midrib region and that this number is intermediate between that of the two parents. It will also be noticed that subsequent to germination the cell number in the linear extension of the midrib region of the cotyledons of all plants is increased. The ultimate sizes of the cotyledons in the two reciprocal hybrids, however, are different from one another, depending on which species has been used as the maternal parent (table 1). Subsequent to increase in cell number in the epidermis, the cotyledon increases in length and breadth. This is accompanied by an increase in average dimensions of the epidermal cells.

As in the later foliage leaves (fig. 3, 4), so in the cotyledon, there is a chronological gradient of differentiation from the distal to the proximal region. The tip differentiates first, then the middle and finally the base, cell enlargement in the latter, as indicated by epidermal studies, ceasing last. In the hybrid whose maternal parent is *A. multiflorus*, enlargement of these cells ceases earlier than in the reciprocal hybrid.

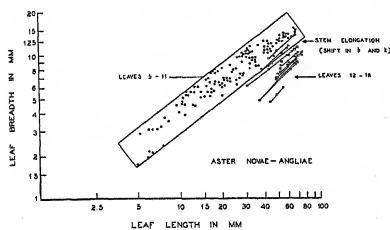
In *Digitalis* (Hill, 1925), F_1 hybrids between species with different types of cotyledons show strong matroclinal tendencies. The influence of the maternal parent is apparent in the size and shape of the cotyledons but does not extend to the true leaves. Since the ratio of the cypsels of *A. novae-angliae* to that of *A. multiflorus* is approximately as 2 to 1, differences in cotyledon size (or even embryo size) in the F_1 may be due in large part to differences in amount of reserve food material present in the ex-endospermous "seeds" of the two parents. Hill offers as a probable explanation of matrocliny in *Digitalis* the relationship of the maternal seed coats to the developing embryo. The subjection of the embryo to differential pressures is suggested as an explanation of the form of the cotyledons—so that their size is in proportion to the size of the seed.



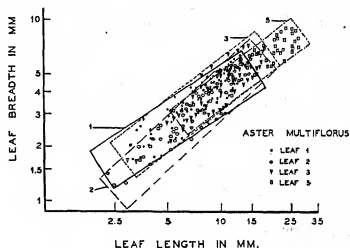
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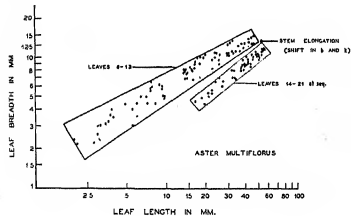
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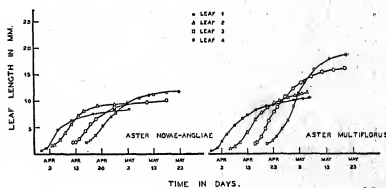
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Fig. 5-10.—Fig. 5. Relationship between cell breadth and leaf breadth in the third leaf.—Fig. 6. Differences in the relative growth rates of the first seedling leaves in *A. novae-angliae*.—Fig. 7. Changes in the ratio of length to breadth and in the relative growth rates of leaves 5-18, incident to stem elongation in *A. novae-angliae*.—Fig. 8. Differences in the relative growth rates of leaves 1-5 in *A. multiflorus*.—Fig. 9. Changes in the ratio of length to breadth and in the relative growth rates of leaves 6-21, incident to stem elongation in *A. multiflorus*.—Fig. 10. A comparison of growth rates in leaves 1-4 of *A. novae-angliae* and *A. multiflorus*.

Vegetative leaves.—(a) Growth of the leaf in relation to growth of the epidermal cells.—Data have been adduced for the first vegetative leaves of the plants. The third leaf has been studied most intensively. It was chosen because it is further removed from the influence of the cotyledons and their high stored food content (the cotyledons absorb the endosperm very early in the development of the embryo). It is also typical of the juvenile leaves in these plants. In figure 2 is given the relationship found between epidermal cell length and leaf length as measured in the midrib region. Since the slope of the line is that of 45°, it is apparent that for the leaf sizes studied the leaf increases in length in direct proportion to the increase in elongation of the epidermal cells. This obtains for both species, *A. novae-angliae* and *A. multiflorus*.

As in the cotyledon, it is of interest to note that cell elongation is not uniform throughout the epidermis. Figure 3 represents three regions in the leaf of *A. novae-angliae* arbitrarily chosen for this study. As shown in this figure, there is a gradient of cellular elongation from the tip to the base, with the epidermal cells in the tip elongating much less rapidly than the leaf as a whole and those in the base elongating more rapidly than the leaf as a whole. There is also a gradient of differentiation extending basipetally from the tip, whose cells cease elongating, mature, and become green prior to any other region in the leaf (this is especially true of the later formed leaves).

Figure 4 shows that essentially the same conditions prevail in the development of the epidermal cells in *A. multiflorus* as is found in *A. novae-angliae*. There is a similar basipetal gradient of cellular differentiation.

In figure 5 are given the relationships between breadth of lamina and average epidermal cell breadth at the broadest part of the leaf in both species. From the graph it is apparent that the leaf as a whole broadens at the same rate as the mean breadth of the epidermal cells increases.

(b) Growth of the entire leaf in relation to the growth of other leaves.—A study of relative growth in the first seedling leaves of *A. novae-angliae* in figure 6 indicates a different k for each of the first few leaves. The fifth and subsequent leaves have a much lower k than the preceding four leaves. There is a tendency for k to stabilize itself in the later leaves (leaves 5 to 10). In figure 7 are given data from the fifth to the eighteenth leaves of the same species. It will be seen that here there is a very definite shift in the ratio of rates of growth in length and breadth in the eleventh and subsequent leaves. This shift in k , initiated approximately in the eleventh leaf, is correlated with the beginning of stem elongation from the rosette. At this same time, as indicated by a progressive change in the levels of the lines, a shift in b may also be noticed. Thus, there is a striking change in the shape index in these leaves as soon as stem elongation begins from the rosette.

A study of figure 8, that of leaves 1 to 5 in *A. multiflorus*, also indicates a marked difference in k in these early leaves; the ratio of the relative growth rates of the subsequent leaves is shown in figure 9. Here it will be noticed that there is a constant k in leaves 6 to 13 but a definite shift in k in leaves appearing later, namely 14 to 21. It is obvious that there has been a change in the ratio of length to breadth—that is, in the shape index (Sinnott, 1927)—in leaves 14 to 21 when contrasted with leaves 6–13—i.e., a definite shift in b in the relative growth formula. It may also be noted that these changes in k and b are associated with the elongation of the stem from the rosette condition, for it will be recalled that the young leaves up to 13 are still a part of the rosette and always remain so, even though the subsequent leaves appear on an elongating axis. The conditions, then, in *A. multiflorus* are similar to those obtaining in *A. novae-angliae* incidental to stem elongation.

The growth rates at which these early leaves develop are interesting and rather significant. In figure 10 on which the data for the two species are presented, the growth rates of comparable leaves on the two species are different. The leaves give typical growth curves, with the grand period of growth within each successive leaf appearing only at the time when that of the preceding leaf is falling off. The progressive increase in size of the successive leaves is also associated with a slightly greater cell number in the epidermis in each leaf over that preceding it.

Up till the present, our study has been confined to the morphogenetical development of the cotyledon and the juvenile leaves in the seedling rosette up through the development of the transitional cauline leaves growing on an elongating axis. It will be recalled that there has been a change in k and b simultaneously with upright growth of the axis from the rosette condition.

It will be of interest to compare the ratios of relative growth rates in leaves produced in the second season's growth—that is, those which have passed a winter in the dormant rosette condition—with those of first year plants.

Information as to the ratio of relative growth rates of the leaves produced during the second growing season was obtained from leaves in the new rosette and the new elongating axis of the same seedlings from which data were obtained the preceding year. It was suspected that the growth coefficient of the successive leaves on the elongating axis would be identical to that of one single leaf studied developmentally. The question is, therefore, whether the developmental pattern of successive leaves in a bud on an elongating axis is the same as that obtained from one single leaf studied throughout all the stages of its individual development. Figure 11 shows that this condition holds. In this figure successive leaves in a young developing bud were studied and the ratios of length to breadth compared with those of succes-

sive developmental stages in a single leaf. It will be seen that the k 's and also the b 's in the two sets of leaves are identical. In *A. novae-angliae* an obvious shift in the very young leaves suggests that the relationship of length to breadth changes in the very early stages of these young leaves. This change will be discussed later in its relation to cellular differen-

tiation in these early leaves. In the older plants a change in b from the rosette to the cauline leaves was also noticed, as was found in the rosette and cauline leaves of the seedlings.

A point of interest to be noted is that the k of the cauline leaves of the second year's growth in each species is identical to that of the cauline leaves (those on the elongating axis) of the seedling of the preceding year—that is, it is now constant. In figure 11 it is important to note the difference in k between the two species. In figure 12, a comparison of the growth coefficient in the developing bud leaves of the hybrids shows it to be intermediate between those of their parents. A shift in k is noticed in the youngest leaves, suggesting a change in the relationships of length to breadth in their early development.

The auxin output obtained by diffusion from these same leaves has also been studied in the whole and in different regions of the same leaf. Figure 13 presents the auxin output from leaves of different weight from a single plant and therefore of different growth stages. The descending curve shows that the auxin output of the leaves is highest in the youngest leaves and least in the oldest. In figure 14 another set of experiments is presented to show the relationship between the auxin output (expressed in auxin units) and the size of the leaf. There is an increase in auxin output from the youngest leaves (less than 5 mm. in length) in the two species and the hybrid. This increase in auxin output is correlated with increase in length of leaf, until a maximum diffusion is reached. Auxin output then decreases as the linear dimension of the leaf increases.

It is obvious that increase in the size of a developing leaf is due either to increase in cell number or increase in cell size or both. Thus the question arises again as to the relationship between leaf length, cell length, and auxin output. Figure 15 presents the relationship between leaf size and epidermal cell size and indicates that in the shortest (youngest) leaves studied, cell size as measured on the epidermis of the midrib is approximately constant, even though the leaf is increasing in length. Hence the leaf is growing in length as a result of cell division. As cell division tapers off when leaves are 5–8 mm. long, cell enlargement becomes the major factor in the increase in length of the leaf. In both species it will be seen that in all leaves 5–8 mm. or more in length the increase in leaf length is in proportion to the increase in epidermal cell elongation. A glance at figures 11 and 12 will show that in leaves over 5–8 mm. long, the breadth increases in direct proportion to the length of the leaf. In figures 13 and 14 it is very interesting to note that the maximum auxin output is obtained from leaves which are approximately 5–8 mm. long. It will be noticed from figures 14 and 15 that cell elongation in the epidermis and decrease in auxin output are clearly correlated. The decrease in auxin output, therefore, seems to take place after cell division in this tissue has ceased and cell elongation begins.

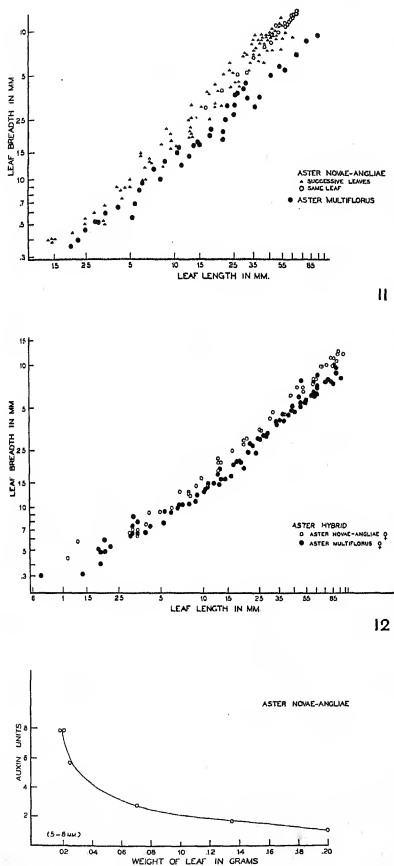
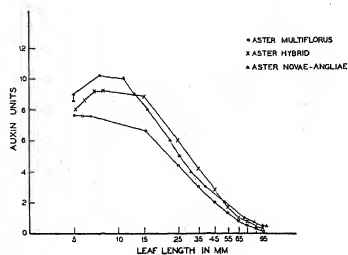
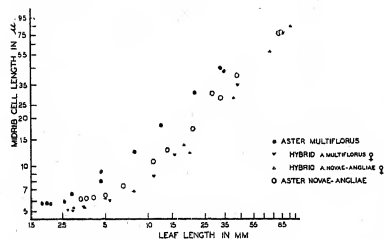


FIG. 11-13.—FIG. 11. Relative growth rates in successive leaves and in ontogenetic stages of the same leaf in *A. novae-angliae* and *A. multiflorus*.—FIG. 12. Relative growth rates in the reciprocal hybrids.—FIG. 13. Auxin output and weight of leaf in *A. novae-angliae*.

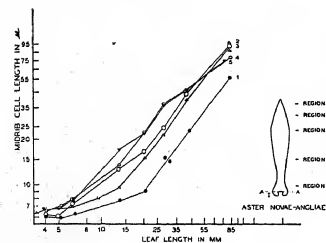
Cell elongation, however, as previously pointed out for the younger leaves of the seedling, does not take place uniformly and at the same time throughout



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Fig. 14-16.—Fig. 14. The relation between size of leaf and auxin output from leaves in *A. multiflorus*, *A. novae-angliae*, and their hybrid. Data based on averages from 300+ leaves.—Fig. 15. The relation between leaf length and midrib cell length in *A. multiflorus*, *A. novae-angliae*, and their reciprocal hybrids. Compare figures 14 and 15 for correlation between decrease in auxin output and cell elongation.—Fig. 16. Differences in cell elongation along the midrib in different regions of the cauline leaf. Note the gradient of differentiation from the tip to the base.

the epidermis of the leaf. In figure 16 it will be noted that cell division in this tissue ceases earlier in the tip than in any other region. Cell elongation in this same tissue is also initiated earlier in that region but is not proportional to the rate at which the leaf as a whole is growing.

In contrast, cell division in the epidermis of the base of the leaf continues longest. This is especially pronounced in the region of the auricles where meristematic activity is greatest and of longest duration. The auricles appear relatively late in the development of the leaf. In the primordium of the auricle—which is at first just a very slight protuberance at the base of the lamina on each side of the midrib—cell division, as is shown by epidermal cell studies, early becomes diffuse and then is followed later by cell enlargement as usual. Table 2 attempts to correlate the amount of epidermal cell division,⁶ the length of the auricles, and the size of the leaf in *A. novae-angliae*. In con-

TABLE 2. A comparison of auricular length and cell number with leaf length in *A. novae-angliae*.

Leaf length in mm.	Auricle	
	Length in mm.	Cell No.
7.5	.08	15
11.3	.112 ^a	21
19.0	.157	25
98.0	3.0 ^b	107

^a Undifferentiated, no veins, non-green.

^b Fully differentiated.

trast, in *A. multiflorus* there is no such basal meristem since auricles are entirely lacking in this species.

In figure 17, the relationship between epidermal cell breadth and leaf breadth is given for the cauline leaves of the two species and their hybrid. In *A. novae-angliae* and the F_1 (*A. novae-angliae* \times *A. multiflorus*) the lamina first broadens without a corresponding broadening of these cells. This indicates cell division which is followed later by cell broadening proportional to the increase in breadth of the lamina. In *A. multiflorus* for the stages studied only cell enlargement is indicated. The cessation of cell division has taken place at a still earlier stage than is indicated on the graph.

It is of interest to consider some of the factors which bring about a greater broadening of the lamina in one region rather than in all regions equally. In figure 18, which is based on data obtained from a series of leaves in a bud growing on the developing axis of *A. novae-angliae*, the breadth of the lamina in the tip, middle, and base is plotted against the number of epidermal cells on an axis perpendicular to the midrib and extending from the midrib to the periphery of the lamina in each region. It can be

⁶ The length of the auricle is designated as that dimension whose axis is parallel to the midrib of the leaf. The number of cells merely denotes the tiers of epidermal cells along this axis whose upper limit is a line A-A in fig. 16.

seen from the figure that the broadest region is made up of a larger number of epidermal cells than is either the tip or the basal region. Cell division in this tissue continues rather longer in that region, giving rise to greater breadth to the leaf upon enlargement of these cells.

That cell division here is of rather diffuse type taking place to some extent in the whole lateral half of the lamina but more particularly in its peripheral region is shown in figure 19. It can be seen that slightly more cell divisions take place in the outer half of the epidermis of the lamina than in its inner half, giving rise to a correspondingly greater number of cells in that region of the lamina.

DISCUSSION.—In the present study emphasis has been placed on the development of the leaf rather than on its origin from the growing axis. This study attempts to analyze the development of form and shape in the leaf with a view to understanding the auxin relationships, which may function in the leaf. No review of the literature of the origin of the leaf primordium need be given here, since a recent survey has been presented (Foster, 1936). The older and scattered literature on the origin and differentiation of tissues in the lamina has also been discussed by Avery (1933) and Smith (1934).

Leaf development in *Aster*, as exemplified by the species studied, is of the generalized sort (Schüpp, 1926).⁷ The primordium undergoes a period of cell division in the formation of the lamina. As the lamina increases in size, diffuse cell division continues throughout the epidermis for a time and is then followed by cell enlargement. However, maturation is not simultaneous in all parts of the lamina; rather, it is clearly of a "basipetal" sort, the tip becoming mature first, then the broader portion of the lamina and finally the base. Growth in the leaf as is indicated by epidermal studies is then interpreted as the integrated activities of cell division and cell enlargement, thereby giving form to the structure. An attempt is made in this paper to analyze the differential growth rates in various segments or regions of a developing leaf. This analysis involves (1) a comparison of these differential rates of growth in the several regions of the leaves studied with those of the leaf as a whole and (2) a comparison of the differential rates of growth in each of two dimensions in the same leaf.

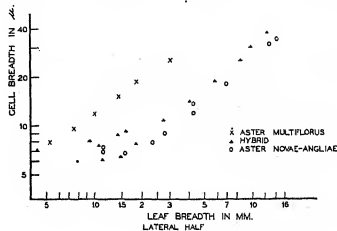
This ratio between the relative growth of a part of an organ to the organ as a whole, or between two dimensions in the same organ, called the growth coefficient (Huxley, 1932, etc.),⁸ and expressed by the

⁷ Schüpp (1926) considers growth in the lamina of a leaf as due to a plate meristem (Plattenmeristem) in which there are two sharply defined directions of cell division: (a) the direction perpendicular to the surface, allowing for leaf expansion, and (b) the direction parallel to the surface, permitting increase in thickness. These plates of cells are clearly visible through the epidermal cells in the very young stages of the leaves studied.

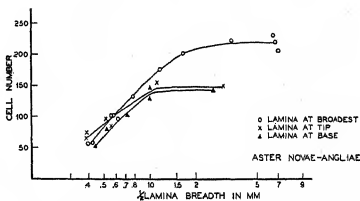
⁸ By relative growth-rate is meant the rate of growth per unit weight—that is, the actual absolute growth-rate

symbol k , is partly responsible for the final shape of the organ. An examination of the growth curves (fig. 10) of *Aster* leaves shows that the grand period of growth takes place at a time after cell division in the outermost tissue of the leaf has ceased and while cell enlargement is manifest. In the seedling, each successive leaf appears when the growth of the preceding leaf is falling off (fig. 10).

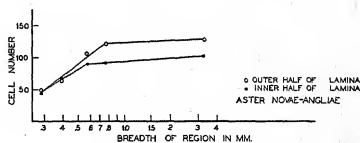
In a cyto-genetical study differences were found in sizes of embryos and achenes in the two parents and their reciprocal hybrids (table 3).⁹ As has been



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Fig. 17-19.—Fig. 17. Relationship between cell breadth and leaf breadth in the cauline leaves of *A. novae-angliae*, *A. multiflorus*, and their hybrid.—Fig. 18. The relation between lamina breadth and cell number in three regions of the blade in *A. novae-angliae*.—Fig. 19. Relation between cell number and breadth of the lamina in *A. novae-angliae*. Note the larger number of cells in the outer half of the lamina.

at any instant divided by the actual size at that instant. (Huxley, 1932, p. 6).

⁹ Random samples gave an average standard deviation of $\pm .021$ mg. for weight per seed.

Further work on the study of reciprocal hybrids has

TABLE 3. Relative achene weight in *Aster*.

<i>A. multiflorus</i> × <i>A. multiflorus</i>	<i>A. multiflorus</i> ♀ × <i>A. novae-angliae</i> (<i>albus</i>) ♂	<i>A. novae-angliae</i> ♀ × <i>A. multiflorus</i> ♂	<i>A. novae-angliae</i> × <i>A. novae-angliae</i> (purple or red)	<i>A. novae-angliae</i> (<i>albus</i>) × <i>A. novae-angliae</i> (<i>albus</i>)	<i>A. amethystinus</i> × <i>A. amethystinus</i> (<i>F.</i>)	<i>A. novae-angliae</i> ♀ × <i>A. amethystinus</i> (<i>F.</i>) ♂	<i>A. multiflorus</i> ♀ × <i>A. amethystinus</i> (<i>F.</i>) ♂
Achene (mgm.)... 2	22	4	43	41	3	44	2

pointed out above, though the cotyledons of these reciprocal hybrids differ greatly in size at maturity, they do not differ in the number of epidermal cells (in length) in their initial stages.

A study of the growth coefficients of the cotyledons has shown them not to be markedly different from the subsequent vegetative leaves. There is a basipetal gradient of differentiation with the tip maturing first and the base last. This is correlated with cellular activity in these regions.

The growth coefficients in the first twenty leaves of the seedlings have been investigated and have yielded interesting results. It is obvious that the growth coefficients or *k*'s, resulting from a logarithmic plotting of length and breadth, are very different in the first 4 or 5 rosette leaves in these plants. There is a tendency, however, for *k* to stabilize itself in the later leaves of the rosette, as is shown in figures 6-9, but as soon as stem elongation begins, the *k* again shifts and the leaves which now result are no longer rosette leaves but cauline or stem leaves. In addition to this shift in *k* there may also be a change in *b*. According to Huxley (1932) the constant *b* is of "no particular biological significance since it merely denotes the value of *y* when *x*=1, that is, the fraction of *x* which *y* occupies when *x* equals unity." As Sinnott (1927) and Kaiser (1935) have earlier disagreed, so the writer disagrees with this statement, as *b* seems indicative of the biological capital—possibly size of primordium—possessed by the leaf even before the relative growth rates are operative.

The subsequently formed leaves on the elongating axis of the seedling continue to have the same length-breadth relationship—i.e., *k* is now constant. This is found to obtain in both species of *Aster* investigated.

As these *Asters* are perennial species, the vegetative parts die down at the close of the growing period. In the early spring a rosette of leaves appears above the level of the ground, but does not send up any shown that initial differences failed to persist. In the seedling resulting from the larger achene of *A. novae-angliae* ♀ mature size was reached earlier, at the end of the first growing season, as compared with the second growing season in the reciprocals resulting from a smaller achene of *A. multiflorus* ♀. The ultimate size reached was found to be the same in the two hybrids from a reciprocal cross (cf. Passmore, 1934), but the limit is reached first by that one which starts with the greater capital, the other attaining that limit only after a longer period of growth. Passmore (1934), in a study of hybrid vigor, has also emphasized the importance of initial size and duration of growth.

elongating axis until the length of day or daily duration of illumination is markedly increased. These new rosette leaves have a very different shape index from the stem leaves of the preceding year. They are much broader per given length so that when length and breadth are plotted against each other logarithmically the resulting curve is on a very different level from that of the cauline leaves of the previous season. The *k* is also different.

In contrast, the cauline leaves growing on the elongating axis of the second season's growth have identical *k* to that of the cauline leaves of the preceding year. In fact, as already pointed out in figures 11 and 12, successive leaves in the terminal bud have exactly the same growth coefficient (*k*) as is found in successive developmental stages of the same leaf—i.e., the *k* has now become stabilized (*k* = 1.0 in *A. novae-angliae*, and *k* = .80 in *A. multiflorus*).

It thus appears that the shift in *k* and *b* in leaves 11 in *A. novae-angliae* and in 14 in *A. multiflorus* are clearly correlated with the elongation of the main axis. This is confirmed by a repetition of the phenomenon during the second year's growth during which the *k* and the *b* change as soon as stem elongation is initiated. Furthermore, elongation from the rosette can be induced artificially with increased illumination.¹⁰

It has been shown that the size and shape of the leaf is correlated with increase in epidermal cell number and epidermal cell enlargement. The shape of the leaf is not the result of differences in cell shape but is due in part to factors limiting the number and direction of the cell divisions in the leaf (Tenopir, 1918) and also to the extent of cell enlargement. In *Aster*, the general form of the leaf is determined early in ontogeny, as was found by Smith in *Acer* and by Avery in *Nicotiana*.

¹⁰ Increased illumination 3 hours per day with a National Carbon Daylight Arc Lamp for 28 days in January induced stem elongation and great vegetative activity in *A. novae-angliae*. It has been shown (Delisle, 1937) that an increase in length of illumination was associated with both a larger output of auxin by the growing tip and stem elongation from the rosette condition. Later, Inge and Loomis (1937) found that internode elongation was dependent upon a constant supply of auxin from the tip. They showed further that in maize, light alone (especially ultra-violet) actually stopped elongation of the first internode, as did decapitation. However, applying auxin at the time of illuminating the seedlings eliminated the inhibiting effect of light on internode elongation. In *Avena* it has been shown (Avery, et al., 1937) that light shortens the first internode of the axis by inhibiting cell division.

In the lamina, cell size in the epidermis is relatively constant in widely diverse areas.

In later stages of development leaf length increases in direct proportion to the increase in cell size as determined by epidermal studies. In the earliest stages, however, the increase in length of the leaf is correlated with the multiplication of epidermal cells. Likewise, the increase in breadth of the leaf is also correlated with cell division and later with cell enlargement in this tissue. In the first few seedling leaves, the breadth of the leaf grows at a faster rate than the length (fig. 6), producing a leaf which is almost round and whose shape index is very different from that of the succeeding leaves. This obtains in both species and the hybrid. In the stem or cauline leaves of the mature plants the leaves in *A. novae-angliae* (fig. 11) grow in breadth at the same rate as in length. In *A. multiflorus* (fig. 11), however, the length increases faster than the breadth, producing a long narrow leaf.

The differences in the breadth of the lamina at the tip, the middle, and the base of the leaf are correlated with epidermal cell number. It was found that cell division continued longer in the epidermal cells of the middle lamina than in the tip or base. This region becomes the broadest portion of the blade. It was also found that most of the epidermal cell divisions occurred in the outer half of the lamina and were of a rather diffuse type.

The auricles in *A. novae-angliae* arise from a portion of the leaf which remains meristematic for a comparatively long time. They appear relatively late in the development of the leaf as a result of diffuse cell division in the base of the lamina on either side of the midrib.

Thus leaf shape is initiated by a "generalized" growth. As pointed out by Avery (1933), the final shape attained by the leaf as a whole may be due in part to a subsequent differential distribution of growth in its various portions—localized growth—and to later growth in one dimension rather than in another in different regions of the leaf—"polarized" growth.

The auxin output of the leaf as measured by leaf weight or by leaf age (or size) is found to increase very definitely at first in the very young stages, to reach a maximum output, then to decrease as leaf length or weight increases. It will be noted in figures 13 and 14 that the maximum output is reached when the cauline leaf is approximately 5-8 mm. in length (.02 gr. in weight) and then decreases thereafter with increase in leaf length. If epidermal changes are indicative of trends in the leaf as a whole, then cell elongation and not cell multiplication is responsible for leaf elongation beyond the 5-8 mm. stage. It would then seem logical to assume that cell elongation and decrease in auxin output are correlated. The relationship, however, between increase in auxin output, previous to the 5-8 mm. stage, and cell division is not as yet at all clear. Decrease in auxin output is also clearly correlated (Delisle, 1937) with stem elongation, but not with cell division.

Here a definite concentration gradient is shown from tip to base of leaf. It is lower at the distal end and successively higher toward the base. The high auxin concentration at the base (Avery, 1935) may not be due entirely to accumulation at the base, but may also be correlated with the greater activity in that region (such as auricle development in *A. novae-angliae* and the hybrid). In a recent paper Thimann and Sweeney (1937) have pointed out, from a study of protoplasmic streaming in epidermal cells in *Avena*, that the action of auxins in promoting growth is exerted not upon the cell wall but upon the cell contents, the protoplasm.

Elongation of the midrib in *Nicotiana*, according to Avery (1935), is due to favorable concentrations of auxin, as growth can be artificially induced by external applications of the auxin paste to the midrib. Avery (1935) suggests that the impetus for leaf development may arise from the main veins due to the accumulation of auxin in them.

In conclusion, it is suggested that the pattern of development in the leaf is directly or indirectly correlated with the distribution and concentration of auxin. Further, as is indicated by relative growth rates, there is found a common pattern of development in successive cauline leaves quite unlike that of the rosette leaves. This is associated with elongation of the axis and changes in auxin content in the apical meristem.

SUMMARY

An analysis of the differential growth rates by the method of Huxley (1932) and Sinnott (1936) in the whole and in various regions of a developing leaf and in successive leaves indicate a relationship between epidermal cell growth and differentiation and leaf size. The size and shape of the leaf are due both to cell number and cell enlargement. The shape of the leaf is not the result of differences in cell shape, but is due in part to factors limiting the number of cells and the direction of cell enlargement. The growth coefficients resulting from a logarithmic plotting of length and breadth of the cotyledons, the seedling leaves, and the later cauline leaves show that there is a basipetal gradient of cellular differentiation in the epidermis. It is suggested that the pattern of foliar development is related to the distribution and concentration of auxin.

From a study of relative growth it was found that successive rosette leaves on the same plant in the two species of *Aster*, *A. novae-angliae*, L., *A. multiflorus*, Ait., and their hybrids, have different relative growth rates and hence different shape indices. In contrast, the successive leaves appearing on the elongating axis have identical relative growth rates and therefore similar shape indices. This is found to hold true in these plants, whether in the first or second season's growth.

It appears that the shifts in k (relative growth rate in the heterogonic growth formula) are clearly correlated with the elongation of the main axis. This

is confirmed by a repetition of the phenomenon during the second year's growth during which the k , at first variable, becomes constant as soon as stem elongation is initiated. Further, elongation from the rosette can be induced artificially with increased illumination.

This uniform pattern of foliar architecture in cauline leaves is invariably associated with elongation of the main axis from the rosette condition and is correlated with differences in auxin conditions in the meristem of the terminal bud.

Cell elongation in the epidermis and decrease in auxin output by the leaf are clearly correlated.

Achene weight and embryo size in the reciprocal hybrids resulting from a cross between these two species of *Aster* differ greatly, depending on the maternal parent. This matrocliny in the embryo of the reciprocal hybrids seems to be due to differences in the amount of reserve food material available for development within the ovary walls of the seed parent.

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FORMATIVE EFFECTS OF RADIATION UPON FERN PROTHALLIA¹

Florence Brown Charlton²

THE WRITER became interested in the relation between quality of radiation and fern gametophyte development while engaged in experiments concerning the effect of quality of radiation upon higher plants, and after translating Klebs' (1916, 1917) classical papers upon the development of fern prothallia. It has been demonstrated repeatedly that the blue end of the daylight spectrum prevents etiolation phenomena in higher plants. Popp (1926), in a careful study in which intensity of radiation was equalized in different regions of the spectrum, was the first to provide accurate evidence that quality of radiation may be a determining formative factor. The work of Klebs, and also that of Stephan (1928, 1929a, 1929b) and Teodoresco (1899, 1929) indicated a similar rôle for the effect of quality of radiation upon fern prothallia. Under red screens the prothallia became filamentous, while under blue screens they developed into plate-like forms.

Experiments carried out by Popp and Brown (1927, 1928, 1936) have shown that ultra-violet radiation from an unscreened mercury arc in quartz in amounts not sufficient to kill or inhibit altogether the growth of seedlings may have marked formative effects. Such radiation for periods as short as 1 to 2 minutes per day will prevent elongation of seedlings kept otherwise in total darkness. Seedlings thus treated are shorter than those receiving 12 hours per day of bright sunlight. The effect of short-wave ultra-violet radiation seems to be an extreme expression of the formative effect of blue, visible radiation. Because fern prothallial cells had responded so markedly to differences in the nature of the visible spectrum in the experiments of the German investigator, Klebs, it was thought that responses to ultra-violet radiation might also be marked. Fern prothallia as experimental material have advantages over seedlings in that they are relatively simpler in structure and may be irradiated directly. Also they may be examined with an ordinary microscope. No teasing or killing and sectioning of material is necessary.

Different fern species, according to Klebs' work, showed minor variations in their reactions to light quality, and all prothallia showed variations in form in response to environmental factors other than qual-

ity of radiation. Hence it seemed advisable and necessary to learn first the reactions of the experimental material of this investigation under the conditions of these experiments to variations in light quality of the visible daylight spectrum and then to proceed with investigations involving the radiation from the quartz mercury vapor lamp.

The writer is much indebted to Dr. Henry W. Popp for valuable suggestions and criticisms during the progress of this work.

MATERIALS AND GENERAL METHODS.—Two fern species were used, *Pteris longifolia*, the principal species used by Klebs and also by Stephan, and *Osmunda Claytoniana*, mentioned briefly by Klebs. Most of the earlier work reported in this paper was carried out with *Osmunda Claytoniana*, while the latter experiments were performed with *Pteris longifolia*. *Pteris longifolia* is a greenhouse form fruiting all year round and producing small brown spores which germinate in daylight three or four days after planting. *Osmunda Claytoniana* is a species growing wild in the mountains of central Pennsylvania. The spores of this species, usually shed in May, are much larger than those of *Pteris longifolia* and contain chlorophyll, which gives them a green color. They germinate within 24 hours after planting and give rise to much larger and more vigorous prothallia than do the spores of *Pteris longifolia*.

The germinating spores and prothallia were grown in Petri dishes, in some cases on the surface of a mineral nutrient solution, but for the most part on a solid medium of the solution in 1 to 2 per cent agar. Benecke's solution (Porter, 1935) was used in all the experiments with *Osmunda*, but in some of the work with *Pteris*, Shive's 3-salt solution (0.16 strength) was used. The Petri dishes were enclosed in light-tight chambers covered by glass screens of known transmission.

The diffused light of the laboratory was the only source of illumination in earlier experiments. In later work this was supplemented by the radiation from a mercury arc in quartz of a Cooper-Hewitt mercury vapor lamp, unscreened and screened by various Corning glass filters. In order to equalize approximately the total intensities of illumination under the screens transmitting daylight, layers of cheesecloth were used to make all total intensities approximate that transmitted by the blue screen, which was about 10 per cent of the incident radiation. To equalize to some extent the intensities under the various screens used with the mercury vapor lamp, the distances of the cultures from the lamp and also the lengths of exposures were varied.

The approximate spectral ranges of the screens used and the transmission of filters in percentage of incident energy, with the mercury vapor arc in quartz as the source, are given in tables 1 and 2.

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Since the author was desirous of learning what types of radiation prevented excessive elongation of cells and encouraged cell division, it was necessary to have "control" plants showing greatly elongated cells and few cell divisions. Actively growing cells or those capable of immediate growth under certain light conditions were necessary. Prothallia, unlike seedlings, would not grow in darkness, and if kept in the dark,

10 to 20 prothallia picked at random from cultures of a hundred or more prothallia. Numerous photographs and drawings according to scale were made.

EFFECTS OF DIFFERENT REGIONS OF THE DAYLIGHT SPECTRUM.—*Osmunda* prothallia grown under the G34 screen invariably developed quite differently from those grown under the blue screen, and the percentage germination was much greater. As germination

TABLE 1. Approximate spectral ranges of screens used.

Coming catalogue number	Description	Approximate spectral range (mμ)
—	Mercury arc (no screen) in air	235-623, 1014+
254	Heat-transmitting filter	1000-3000
990	H. R. clear Corex D, 2 mm.	250-720+
306	Noviol O	389-720+
597	G586A red purple ultra ^a	approx. 300-436 + 5% in red end
	G34	529-720+
	G403ED blue glass	374-685

^a Because the G586A screen transmitted to some extent in the red end of the spectrum, with certain cultures a 7.5 per cent CuSO₄ liquid screen 2 cm. thick was superimposed on the G586A screen to absorb this long-wave radiation.

TABLE 2. Transmission of filters in percentage of incident energy with mercury vapor arc in quartz as source.

Wave lengths Angstroms	Corex 2 mm.	Noviol O	G586A	2 cm. 7.5 % CuSO ₄ plus G586A
5791	94.86	89.66	—	—
5770	94.86	89.66	—	—
5461	87.06	86.80	—	—
4359	91.62	52.06	18.64	18.25
4078	79.34	—	—	—
4047	81.53	27.05	15.56	14.76
3907	—	25.47	—	—
3656	80.83	—	—	—
3342	59.02	—	37.45	6.66
3132	—	—	7.05	0.007
3126	—	—	7.05	—
3022	68.55	—	10.91	0.0011
2967	49.15	—	—	—
2894	42.85	—	—	—
2857	32.99	—	—	—
2804	40.14	—	—	—
2753	29.88	—	—	—
2645	16.56	—	—	—
2537	0.11	—	—	—

would not respond within 24 or 48 hours to short exposures to light. Hence "dark" cultures could not be used as controls or for comparative purposes as in the work with seedling plants. Instead, prothallia developed under the G34 screen in diffused daylight were used, since these being exposed to only the red end of the spectrum, were found to be without exception filamentous forms with elongated cells, dividing infrequently, but capable of responding within a relatively short time to certain types of radiation.

The experiments were run in duplicate and repeated from one to several times. Length measurements were made in ocular micrometer units on from

began under the G34 screen, the first rhizoid emerged through the broken spore wall. Eventually (2 to 3 days) a short, thick, 2-3-4-celled filament developed. The tip cell of this filament then became increasingly elongated. A cross wall appeared in it (fig. 1, D). Further development resulted in a long filament of cells (fig. 1, E, F), cross walls appearing more frequently under strong illumination than under weak illumination. Such prothallia retained their strictly filamentous nature for more than a year (until the culture was discontinued) in the diffused light of the laboratory under G34 glass.

Under the blue screen, very early, sometimes the second day, and commonly the third day after planting, the germ tube ceased to be a filament of cells. Lengthwise divisions appeared in the second or third cell from the base of the filament (fig. 1, A). The tip cell never elongated, but divided lengthwise. Further divisions of the essentially isodiametric cells occurred in various directions, resulting in a plate-like prothallium (fig. 1, B, C). In the course of a year's development no elongated cell ever developed under the blue screen, even when the light intensity was considerably reduced and far below that which caused the development of elongated cells under the G34 screen. Cell divisions occurred more frequently under the blue screen than under the G34 screen.

The behavior of *Pteris* under conditions similar to those described for *Osmunda* was very much like that of *Osmunda*. However, the *Pteris* spore took longer to germinate (3 to 4 days) and did not produce as large or vigorous a prothallium. The filamentous form of the prothallium did not change so readily into the plate-like form. This may be correlated with the fact that the *Pteris* spore is smaller than the *Osmunda* spore and lacks chlorophyll. Hence food for metabolic processes would not be as abundant in

the *Pteris* spore and young prothallium. An accumulation of food seems to be necessary for cell division. Under the blue screen, *Pteris* spores, like *Osmunda* spores, eventually developed at the tip of a several-celled germ tube, a plate-like mass of cells (Plate 1, fig. 2, 3). Under the G34 screen, the prothallia remained filamentous (Plate 1, fig. 1). In *Pteris*, however, the filaments sometimes remained one-celled for weeks and never became more than few-celled, the individual cells being extremely elongated.

The development of *Pteris* and *Osmunda* under the blue screen resembled that under window glass or under a Noviol O screen, except that the cells under the blue screen were more nearly isodiametric. The blue end of the spectrum, then, produced more nearly "normal" prothallia than the red end.

The contrasting effects of the blue and red ends of the daylight spectrum on growing prothallial cells were demonstrated perhaps more strikingly in another manner. Filamentous plants developed under the G34 screen were placed under the blue screen. Conversely, plate-like prothallia developed under the blue screen were placed under the G34 screen. Within 36 to 60 hours after a 5-weeks-old filamentous *Osmunda* prothallium produced under the G34 glass was placed under a blue screen, cell divisions at right or acute angles to the lengthwise axis of the cell were noted in the tip cells of the filament (Plate 1, fig. 6, 7). This also occurred if G34 filaments were put under window glass. Further development resulted in a plate-like mass of cells (Plate 1, fig. 8). The control filaments, continuously under the G34 screen, remained strictly filamentous (Plate 1, fig. 4, 5). If, on the other hand, 5-weeks-old prothallia which had developed under the blue screen were placed under the G34 screen, elongation of cells along the growing margins of the prothallia occurred. This was not apparent, however, in 36 hours. The reaction to red light of the cells developed in blue light was not as rapid as was that to blue light of cells developed in red light. Even after 7 days no effects were apparent. By the end of 12 days, however, filaments were growing out from the formerly smooth growing face of the prothallium (Plate 1, fig. 9, 10, 11), and after 54 days, these filaments had reached considerable length (fig. 2).

Prothallia which had been transferred from the red end of the spectrum to the blue end, and which consisted of filaments of cells with terminal plates of cells developed under the blue screen, after 14 days under the blue screen were returned to the original light conditions. Similarly, those which had been transferred from the blue to the red end of the spectrum, and which consisted of plates of cells with filamentous outgrowths developed during the period under the red screen, after 14 days were placed again under the blue screen. Once again the growing cells reacted to the change in light quality. Plates of cells developed in blue light at the ends of filaments previously grown in red light gave rise to filaments of elongated cells when put under the G34 screen again (fig. 3, A), and

filamentous outgrowths of elongated cells which had developed under the G34 screen on plate-like prothallia previously grown under the blue screen gave rise to plates of cells when placed under the blue screen again (Plate 1, fig. 12, and fig. 3, B). *Pteris* fan-shaped prothallia also developed filamentous outgrowths if placed under the G34 screen.

EFFECTS OF VISIBLE, INFRA-RED, AND LONG-WAVE ULTRA-VIOLET RADIATION FROM THE MERCURY VAPOR ARC.—There was particular interest in long-wave ultra-violet radiation since its effects on plants, probably because not striking, have been a subject of much controversy. Although some have claimed that it causes plants and seedlings to be larger, particularly longer, than normal, such an effect could never be confirmed on turnip or other seedlings grown by Popp and Brown (1928, 1936). Under the experimental conditions available, it is unfortunate that a high intensity of such radiation, to the exclusion of other types, could not be provided. The G586A screen alone, the G586A screen in combination with a CuSO_4 screen, and a screen transmitting only infra-red were used, with the mercury arc as the source of radiation. Thus, the effect of predominantly long-wave ultra-violet radiation with infra-red radiation, that of ultra-violet and near ultra-violet alone, and that of infra-red alone could be compared. For experiments with

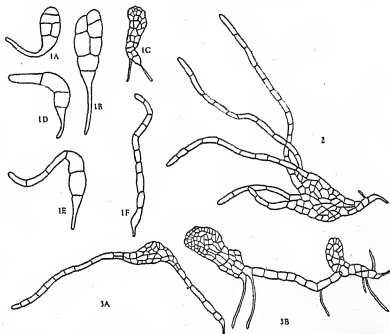


Fig. 1-3.—Fig. 1. *Osmunda Claytoniana* prothallia developed under opposite ends of the visible spectrum. A, B, C, developed under blue screen; D, E, F, developed under G34 screen. A and D, 7 days old; B and E, 12 days old; C and F, 29 days old. C and F $\times 125$.—Fig. 2. *Osmunda Claytoniana* prothallium grown 31 days under blue screen, followed by 54 days under G34 screen. Plate-like development was replaced by filamentous growth under the G34 screen.—Fig. 3. *Osmunda Claytoniana* prothallia. A, grown 31 days under G34, followed by 14 days under blue, followed by 31 days under G34 screen; B, grown 31 days under blue, followed by 14 days under G34, followed by 31 days under blue screen. In each case the filamentous type of growth was obtained under the G34 screen (red end of the spectrum) and the plate-like growth under the blue screen.

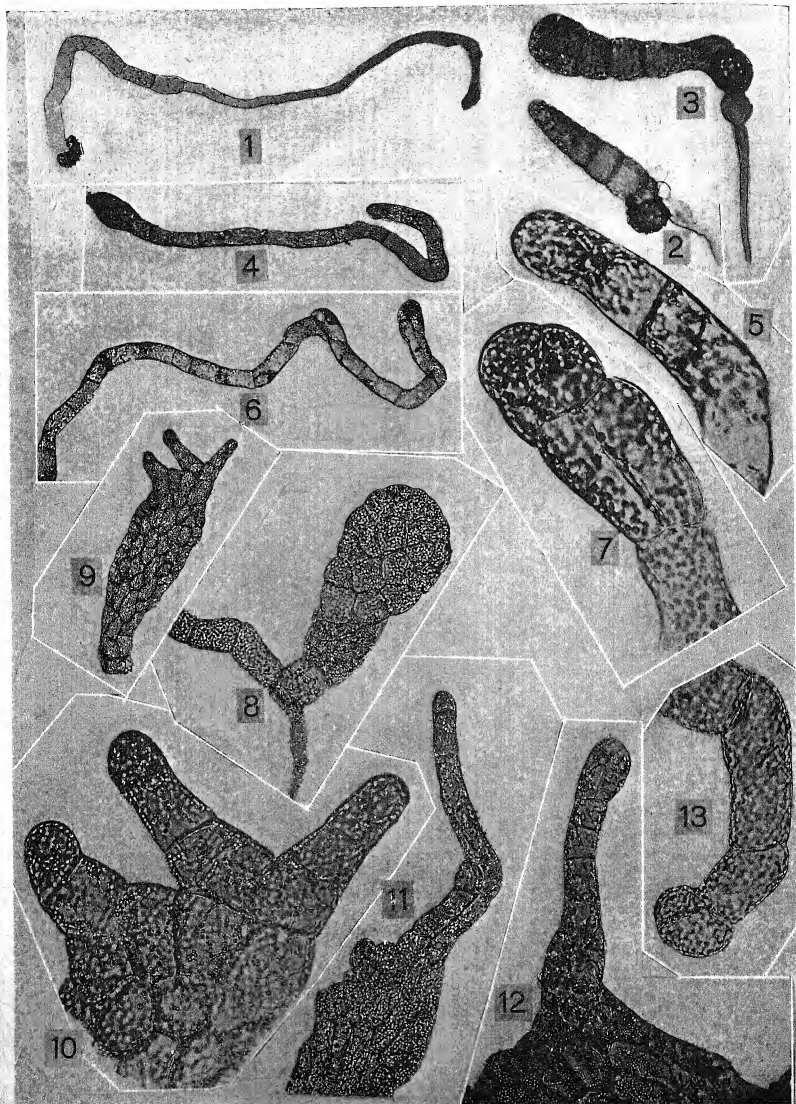


TABLE 3. Effect of mercury arc on *Pteris* spores and prothallia grown in daylight under G34 glass.

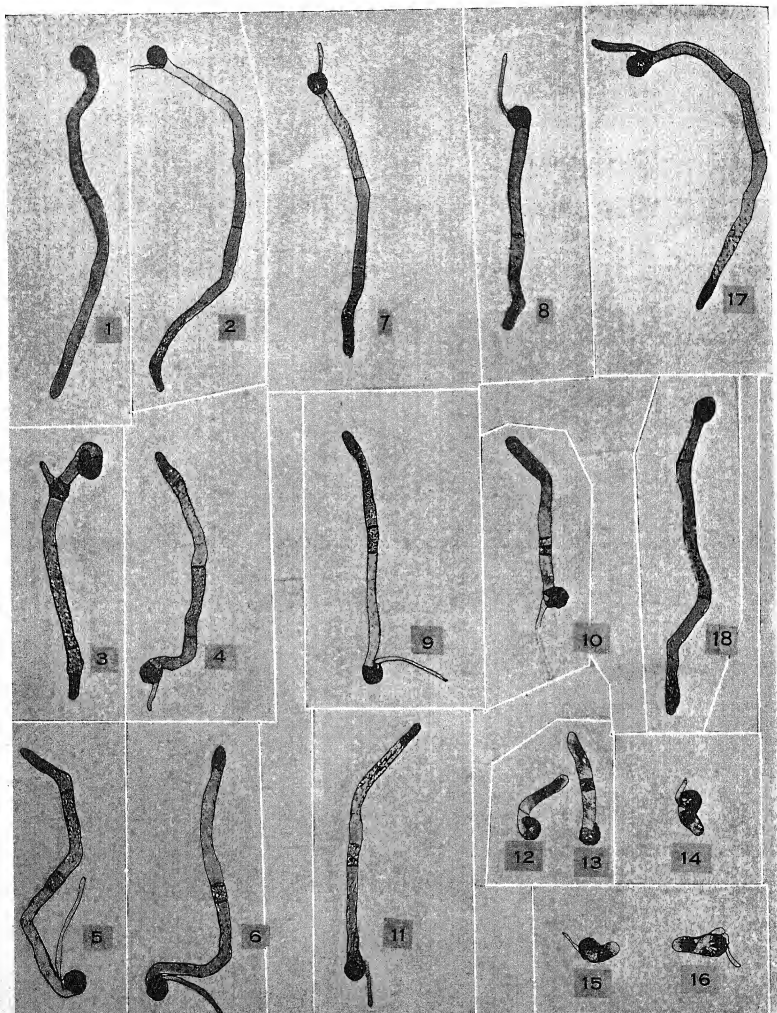
	Av. length filaments (micr. units)		Av. no. cells per filament		Av. length cells (micr. units)	
Series I.						
Age of filaments	10 days	16 days	10 days	16 days	10 days	16 days
No. of daily exposures given	9	15	9	15	9	15
Group 1—Daily exposures for 30 min. at 75 cm. distance from arc						
A. Tinfoil screen	52.6	62.6	2.2	2.9	23.9	21.6
B. Heat-transmitting screen	45.8	62.0	2.9	3.6	15.8	17.2
C. G586A screen	30.9	56.4	3.2	4.7	9.7	12.0
D. G586A and CuSO ₄ screens	36.0	53.1	4.1	5.1	8.8	10.4
Group 2—Daily exposures for 30 min. at 75 cm. except G, 15 min. at 75 cm.						
A. Tinfoil screen	52.7		2.7		19.5	
E. G34 screen	45.6		2.3		19.8	
F. Blue screen	35.5		3.2		11.1	
G. Noviol O screen	34.7		3.1		11.2	
Series II.						
Age of filaments	9 days	15 days	9 days	15 days	9 days	15 days
No. of daily exposures given	7	13	7	13	7	13
Group 1—Daily exposures as above, but increased to 1 hour after 9 days						
A. Tinfoil screen	47.3	58.2	1.9	2.8	22.0	20.8
B. Heat-transmitting screen	50.0	66.3	2.0	3.2	24.5	20.7
C. G586A screen	40.3	54.4	3.2	4.4	11.6	12.4
D. G586A and CuSO ₄ screens	39.8	56.0	3.3	4.0	12.1	14.0
Group 2—Daily exposures as above						
A. Tinfoil screen		56.7		2.6		21.8
E. G34 screen		54.9		2.6		21.1
F. Blue screen		46.9		2.9		16.2
G. Noviol O screen		47.5		3.3		14.4
Series III.						
Age of filaments	12 days		12 days		12 days	
No. of daily exposures given	7		7		7	
Daily exposures for 2 hours at 50 cm. exc. B, 2 hrs. at 75 cm.						
A. Tinfoil screen	40.0		1.5		26.7	
B. Heat-transmitting screen	60.1		3.2		18.8	
D. G586A and CuSO ₄ screens	38.7		4.8		8.1	
G. Noviol O screen	40.0		5.0		8.0	

the visible spectrum of the arc, the G34, blue, and Noviol O screens were used.

Pteris spores were germinated under the G34 screen in daylight and given daily exposures to the mercury arc. By means of tinfoil partitions, each culture was divided into four sections. During irradiations one-fourth of each culture was covered by tinfoil (i.e., received no radiation from the lamp), and each of the

other sections was covered by a different one of the above mentioned screens. Thus a single culture had one section covered by tinfoil, receiving, therefore, no mercury arc radiation, one section receiving the mercury arc radiation through the heat-transmitting screen, one, the radiation through the G586A screen, and one, the radiation through the G586A screen plus the CuSO₄ screen. Similarly, another culture had the

Plate 1.—Fig. 1-13.—Fig. 1 to 3, *Pteris longifolia* prothallia developed under opposite ends of the visible spectrum; 1, grown under G34 screen; 2, 3, grown under blue screen; all 14 days old; 1 × 75; 2, 3 × 150. Fig. 4-13, *Osmunda Claytoniana* prothallia. Fig. 4 to 7 show effect of replacing G34 screen by blue screen for 36 hours; 4, 5, controls (under G34 continuously); 6, 7, show cell walls developed at acute and right angles to lengthwise axis of filament when G34 screen was replaced by blue screen for last 36 hours of growth. Fig. 8 to 11 show prothallia 46 days old; 8, shows the tip of a filament grown for 34 days under G34 screen after it had grown subsequently for 12 days under the blue screen; 9, 10, 11, show plate-like prothallia giving rise to filamentous outgrowths after being 34 days under blue screen followed by 12 days under G34 screen. Fig. 12, grown 34 days under blue, followed by 14 days under G34 screen during which plate-like development ceased and filamentous growth ensued, followed by 2 days under blue screen again, during which filamentous growth was checked and cell divisions at oblique and right angles initiated plate-like growth again. Fig. 13, a filament grown under G34 screen showing a lengthwise cell division after several 15 min. daily exposures to the mercury vapor arc screened by Noviol O glass. Fig. 9, ×60; 4, 6, ×80; 8, 11, 12, ×120; 10, ×240; 5, 7, ×335; 13, ×450. In each case the filamentous type of growth was obtained under the G34 screen (red end of the spectrum) and the plate-like growth under the blue screen.



four sections covered by tinfoil, blue screen, Noviol O screen, and G34 screen. By these means more nearly identical growing conditions, except for the supplementary illumination, were given the prothallia compared than would have been possible if they were grown in separate dishes. The prothallia, except for the exposures to the mercury arc, were kept in diffuse daylight under G34 screens. *Osmunda* filamentous prothallia five months old were also exposed for short periods to the visible spectrum of the mercury arc as transmitted by the Noviol O screen.

The visible spectrum of the mercury arc in which the shorter wave lengths predominate was found to affect the cells of fern prothallia in a manner similar to that of the blue end of the daylight spectrum. Five-months-old *Osmunda* filaments which had been kept under the G34 screen in daylight throughout their development were given daily exposures to the mercury arc covered by Noviol O glass. Several daily exposures of from 15 minutes to several hours caused lengthwise cell divisions in the tip cells of the filaments (Plate I, fig. 13). Similar exposures to the arc screened by G34 glass caused no such developments. The prothallia remained strictly filamentous. It is assumed that the short visible waves of the mercury arc spectrum produced the lengthwise cell divisions, since the ultra-violet portion was screened out in both cases. *Pteris* spores germinated and allowed to develop under the G34 screen in daylight were given daily exposures at 75 cm. through Noviol O, through blue, through G34, and through heat-transmitting screens. The controls were covered with tinfoil screens. The results (table 3, Group 2 of Series I and II, and Series III) indicate that the blue light of the mercury arc spectrum caused the cells to be shortened and increased the number of cell divisions. Filaments screened by tinfoil or by G34 glass which transmits only long-wave visible radiation were longer, and the cells composing the filaments were fewer in number and more elongated than those screened by the blue or Noviol O glasses and therefore exposed to the blue end of the spectrum. Since the Noviol O screen does not transmit ultra-violet radiation, and infra-red radiation as transmitted by the heat-transmitting screen was not effective, the shortening of cells and increased number of cell divisions may be ascribed to the blue end of the visible spectrum. No lengthwise cell divisions were noted, however. The effect of the arc screened by Noviol O glass for 2-hour daily periods at 50 cm. distance from the arc was especially pronounced.

That cell length and cell division were affected by long-wave ultra-violet radiation is indicated by the data recorded in table 3 and by photographs in Plate 2. Those filaments under the G586A screen or under that screen in combination with the CuSO_4 screen (Plate 2, fig. 3, 4, 5, 6) developed shorter cells and more of them than those subjected not at all to the mercury arc spectrum or only to the infra-red portion thereof (Plate 2, fig. 1, 2). The prothallia exposed only to the infra-red radiation of the lamp resembled those receiving no radiation at all from it. There is some indication that a high temperature, as produced under the heat-transmitting screen, may affect cell length and cell division in prothallia as compared with the controls under the tinfoil screen. The effect, however, is more than compensated for if short-wave radiation is also present, as indicated by a comparison of plants under the heat-transmitting screen with the G586A and G586A plus CuSO_4 plants. Prothallia receiving long-wave ultra-violet radiation resembled those receiving the blue end of the visible spectrum of the lamp (table 3) or those grown in weak daylight (Plate 2, fig. 11). There was no indication that long-wave ultra-violet radiation had any tendency to cause increased growth in length.

EFFECTS OF THE UNSCREENED MERCURY ARC.—The reactions of fern prothallia to the radiation of the unscreened mercury arc are extremely pronounced. One to three-months-old *Osmunda* filaments developed in daylight under G34 glass were given daily exposures to the unscreened arc. Otherwise they were kept under the G34 screen in daylight. At 50 cm. distance from the arc, 5-minute, 2-minute, 1-minute, 30-second, 15-second, 10-second, and 5-second daily exposures injured and killed the filaments. A single exposure as short as 1 minute was lethal. Only 4 or 5 daily 5-second exposures were necessary to cause death. A single 30-second exposure injured, but did not kill the filaments. A single 15-second or 5-second exposure retarded cell development. This was rather conspicuously demonstrated by substituting the blue screen for the G34 screen over control plants as well as over exposed plants after the exposure to the unscreened mercury arc. The blue light caused lengthwise cell divisions to appear in the tip cells of the control filaments within two days. They eventually appeared in the filaments exposed to the mercury arc, but not until several days after they had appeared in the control filaments. After 7 days no cell divisions had appeared in filaments given a single 30-second exposure to the mercury arc. At 100 cm.

Plate 2.—Fig. 1-18.—*Pteris longifolia* 10 days old grown either in daylight under the G34 screen or in weak daylight, and given supplementary daily exposures to the mercury vapor arc. Fig. 1 to 6 show the effect of long-wave ultra-violet radiation upon prothallia grown in daylight under the G34 screen; 1, without supplementary radiation (control); 2 to 6, given one-half hour per day mercury arc at 75 cm. distance; 2, through heat-transmitting screen; 3, 4 through G586A plus CuSO_4 screens; 5, 6, through G586A screen. Fig. 7 to 16 show the effect of the unscreened arc. Fig. 7, 8, grown in daylight under G34 screen plus 5- and 15-second daily exposures, respectively, at 100 cm. (compare with Fig. 1 control). Fig. 9 to 16, grown in weak daylight; 11, without supplementary illumination (control); 9, 10, 14, given 5-second, 15-second, 2-minute daily exposures, respectively, at 100 cm.; 12, 13, given 5-second daily exposures at 50 cm.; 15, 16, given 15-second daily exposures at 50 cm. Fig. 17 to 18 show the effect of the mercury arc screened by Correx D glass upon prothallia grown in daylight under the G34 screen; 17, given 15-second daily exposures at 86 cm.; 18, given 15-second daily exposures at 50 cm. Compare 17, 18, with fig. 1 control. All photographs $\times 100$.

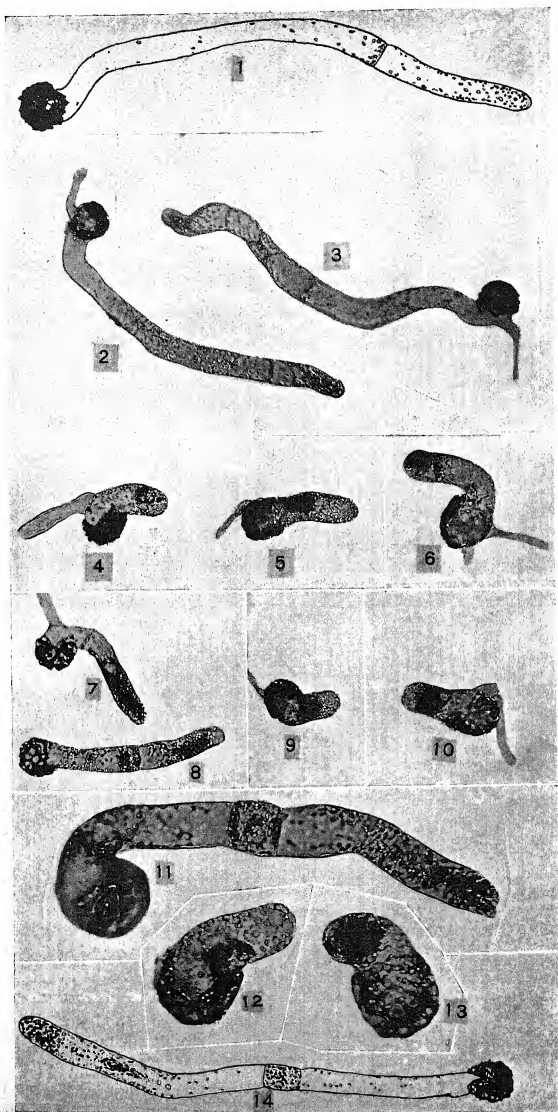


TABLE 4. Effect of unscreened arc and arc screened by Corex D glass upon *Pteris* spores and prothallia.

Distance from arc	Unscreened arc				Arc screened by Corex D			
	50 cm.		100 cm.		50 cm.		86 cm.	
Length of exposure	Av. length fil. (micr. units)	Av. no. cells	Av. length fil. (micr. units)	Av. no. cells	Av. length fil. (micr. units)	Av. no. cells	Av. length fil. (micr. units)	Av. no. cells
Plants grown in daylight under G34 screen, and given 9 daily exposures								
Series I. Cultures 10 days old								
A. No exposure	51.9	2.5	52.0	2.5	48.6	2.8	37.3	2.3
B. 5 minutes	—	—	—	—	5.8	1.1	23.8	2.8
C. 2 minutes	—	—	2.7	1.0	—	—	—	—
D. 15 seconds	4.6	1.0	18.2	2.5	32.8	3.2	33.0	3.4
E. 5 seconds	—	—	35.4	2.4	33.2	2.9	—	—
Plants in weak daylight, given 7 daily exposures								
Series II. Cultures 10 days old								
A. No exposure	45.1	3.4	47.0	3.6	70.6	2.2	55.3	1.9
B. 5 minutes	—	—	—	—	11.2	1.9	28.3	2.0
C. 2 minutes	—	—	4.3	1.0	—	—	—	—
D. 15 seconds	6.7	1.0	23.8	2.5	61.4	2.2	46.8	2.0
E. 5 seconds	17.9	2.6	39.5	3.7	60.8	2.3	52.6	2.0
Plants in daylight under G34, given 7 daily exposures								
Cultures 22 days old								
Given 19 daily exposures				Given 19 daily exposures				
A. No exposure	40.8	3.9	45.4	3.8	140.0	3.0	90.0	3.0
B. 5 minutes	—	—	—	—	10.6	1.4	41.0	3.7
C. 2 minutes	—	—	—	—	—	—	—	—
D. 15 seconds	—	—	23.7	2.8	116.7	3.5	—	—
E. 5 seconds	15.7	2.8	38.2	3.9	127.0	4.3	—	—

distance from the arc, a distance to which the shortest rays reaching the 50 cm. distance do not penetrate, exposures of 8 minutes, 2 minutes, 1 minute, and 30 seconds injured and killed the filaments, the 8-minute filaments showing distinctly disorganized cells after 5 daily exposures, and the 30-second ones, after 8 exposures. A 15-second or 5-second daily exposure for 4 days did not cause prominent injury. Such prothallia, however, failed to react to daylight subsequently by developing lengthwise cell walls. Controls not exposed to the mercury arc showed such lengthwise cell divisions when put in diffused daylight. Neither 5-second exposures at either 50 cm. or 100 cm. distance, nor even exposures as short as 1 second ever stimulated the development of lengthwise walls, or, on the other hand, of longer cells.

Pteris spores were germinated under the G34 screen in daylight and also germinated in very weak daylight (in the laboratory under eight layers of cheesecloth). Both conditions caused the development of elongated, few-celled filamentous prothallia (Plate 2, fig. 1, 11), but the weak daylight induced shorter

cells and more frequent cell divisions than daylight as transmitted by G34 glass. The cultures were divided by means of tinfoil partitions into four sections and given short daily exposures to the unscreened mercury arc. During irradiations, one-fourth of the culture was covered by tinfoil (i.e., received no radiation from the lamp), and each of the other sections was given a certain short exposure to the unscreened arc, being otherwise covered with tinfoil while under the lamp.

Two-hour exposures of the dry spores at 50 cm. distance from the lamp failed to prevent germination. At this same distance 2-minute, and 15-second daily exposures of spores on agar were given for 12 days. Spores of the 2-minute sections never germinated. Those of the 15-second sections germinated, and short, thick, one-celled germ tubes, each with a thickened primary rhizoid developed, but the plants soon died. At this time the control plants which had been protected by tinfoil while under the lamp, and which were now greatly elongated few-celled filaments similar to those of Plate 1, fig. 1 or fig. 11, were given a

Plate 3.—Fig. 1-14.—*Pteris longifolia* prothallia 10 days old grown either in daylight under a G34 screen or in very weak daylight, and given daily supplementary exposures to the mercury vapor arc. Fig. 1 and 14, controls, without supplementary illumination; 1, grown under G34 screen; 14, in very weak daylight. Fig. 3, 5, 6, grown under G34 screen and given supplementary exposures to the mercury arc screened by Corex D glass; 3, given 5-minute daily exposures at 86 cm.; 5, 6, given 5-minute daily exposures at 50 cm. Fig. 2, 4, 7-13, given supplementary daily exposures to the unscreened arc; 2, 11, 15-second exposures at 100 cm.; 4, 12, 13, 2-minute exposures at 100 cm.; 7, 8, 5-second exposures at 50 cm.; 9, 10, 15-second exposures at 50 cm. Fig. 11, 12, 13, $\times 400$; all others $\times 200$. For more detailed explanation see text.

single 2-minute exposure. By the following day all were dead. The whole experiment was repeated, with the addition of a 5-second section in the culture. The first exposure was not given until the third day after the spores were planted. Germination was beginning. The results for the 2-minute and for the 15-second sections (Plate 2, fig. 15, 16, and Plate 3, fig. 9, 10) were similar to those described above for similar sections. The 5-second plants, however, were still alive after 22 days and 19 exposures. They consisted of short, thick, 2-3-4-celled germ tubes with thick primary rhizoids (Plate 2, fig. 12, 13, and Plate 3, fig. 7, 8). In the first two columns of table 4 are given measurements for lengths of filaments and number of cells in each filament, of germinating spores exposed to the unscreened mercury arc at a 50-cm. distance.

Cultures similar to those just described were grown, except that the distance from the mercury arc was increased to 100 cm. The 2-minute prothallia were markedly retarded as compared with the control plants and after 4 daily exposures looked similar to the 15-second, 50-cm. plants (Plate 2, fig. 14, 15, 16, and Plate 3, fig. 4, 9, 10, 12, 13). After 9 daily exposures, the cell contents were disintegrating, and all plants were dead after 12 exposures. The 5-second and 15-second sections of the cultures, on the other hand, were healthy growing ones, with filaments similar to the controls, definitely shorter, however, and the 15-second plants shorter than the 5-second ones (Plate 2, fig. 7, 8, 9, 10, and Plate 3, fig. 2, 11). In the third and fourth columns of table 4 are recorded measurements of filaments given exposures to the unscreened arc at the 100-cm. distance. There, also, a comparison is afforded between cultures placed at 100 cm. from the mercury arc and those placed at 50 cm. from the arc. The less marked effects of the radiation at the greater distance are easily noted.

EFFECTS OF THE MERCURY ARC SCREENED BY COREX D GLASS.—The Corex D screen transmits short-wave ultra-violet radiation down to 250 m μ . The percentage transmission of incident energy is considerably lowered in the short-wave ultra-violet region. Radiation through this screen caused effects similar to but not as pronounced as those caused by the unscreened arc when the total energy values were approximately equalized.

Osmunda filaments four months old, which had developed in daylight under G34 glass were given daily exposures to the mercury arc screened by Corex D glass at distances of 86 cm. and 50 cm., respectively, from the mercury arc. At 86 cm. the total intensity of the radiation was approximately equal to that at 100 cm. from the unscreened arc. Single exposures of 1 hour at 86 cm. caused subsequent death of cells noticeable several days later. Single exposures of one-half hour, 15 minutes, or 10 minutes did not kill filaments but retarded their growth. The tip cells did not show lengthwise divisions if put in white light, while those of control plants did. Shorter single exposures of 5 minutes, 2 minutes, 1 minute, 30 seconds,

15 seconds, and 5 seconds did not cause marked injury, nor did they in any noticeable manner stimulate growth. Fifteen-minute daily exposures for 10 days, however, injured and killed such prothallia. Four daily exposures of 1 minute, 30 seconds, 15 seconds, or 5 seconds, however, did not affect the prothallia. The tip cells, like those of control filaments, subsequently divided in a lengthwise direction when placed in daylight. At 50 cm. distance 1-minute or 30-second daily exposures for several (4 to 7) days caused injury. The tip cells of the filaments did not react subsequently to daylight by dividing lengthwise. Exposures as short as 5 seconds, however, did not prevent this reaction.

Pteris spores were germinated and the young prothallia grown under the G34 screen. Daily exposures to the mercury arc screened by Corex D glass were given at 86 cm. and at 50 cm. from the mercury arc. Five-minute, 2-minute, 15-second, and 5-second daily exposures were given to different sections of the same culture. At 86 cm. distance, after 4 daily exposures, beginning with the day after the spores were planted, the 5-minute section showed somewhat shortened tubes, more markedly shortened ones after 6 and 9 exposures (Plate 3, fig. 3). However, the filaments continued to grow. After 19 exposures, this culture showed short, thick, few-celled germ tubes. The 15-second (Plate 2, fig. 17) and 5-second sections were similar to the control which had received no mercury arc radiation. At 50 cm. distance from the arc, the 5-minute plants, in contrast to the 86-cm., 5-minute plants, after 4 daily exposures showed only slight signs of development. After 9 exposures, very short germ tubes had emerged from the spore cases (Plate 3, fig. 5, 6). The plants looked similar to the 50-cm., 15-second unscreened arc ones (Plate 3, fig. 9, 10), or to the 100-cm. 2-minute unscreened arc ones (Plate 3, fig. 4). After 12 exposures 1-celled or 2-celled germ tubes were present, but the protoplasm looked granular and injured. Two-minute exposures for 7 days also stunted the germ tubes. Fifteen-second (Plate 2, fig. 18) and 5-second prothallia, even after 20 exposures, were similar to controls, except that the filaments were very slightly stunted. In the last four columns of table 4 are recorded measurements of plants exposed to the mercury arc through the Corex D screen at 86 cm. and at 50 cm. distance.

DISCUSSION.—Observations on great numbers of fern prothallia grown under the conditions stated leave no doubt in the mind of the writer that the quality of radiation to which the growing prothallial cell is subjected profoundly affects the nature of its development, particularly the amount of elongation and the frequency and direction of cell division. Such effects in turn modify the configuration and form of the multicellular prothallium.

Of particular interest were the findings leading to the conclusion that long-wave ultra-violet radiation caused responses opposite to those included in the phenomenon of etiolation. In this respect the effects of this kind of radiation are similar to those of the

next longer wave lengths—namely, those of the blue end of the visible spectrum—and strikingly different from those of the still longer rays of the red end of the visible spectrum and of infra-red rays. The long-wave ultra-violet region of the mercury arc as transmitted by either the G586A screen or the G586A screen in combination with CuSO_4 screen did not have extremely pronounced effects in the intensities available. However, it did cause definitely shorter cells and an increased number of cell divisions as compared with radiation minus these short wave lengths. Prothallia subjected to long-wave ultra-violet radiation resembled those subjected to a weak intensity of radiation from the mercury arc as transmitted by the blue screen, or to very weak daylight. The effect of this region of the spectrum on prothallia resembles its effect on seedlings, as shown by Popp and Brown (1928, 1936); it has a stunting effect on elongation.

The short-wave ultra-violet region of the mercury arc, containing wave-lengths shorter than those occurring in sunlight, is extremely injurious to fern prothallia. However, in almost momentary doses which were not sufficient to interfere altogether with growth, this type of radiation was demonstrated to prevent to a very marked degree cell elongation. The shorter the wave lengths included in the radiation, the more noticeable was this effect as shown by the differences in response of prothallia exposed to the unscreened arc as compared with those irradiated under Corex D glass or under the G586A screen, and as shown by the effect of increasing distances from the arc with any given range of ultra-violet radiation. A comparison of the photographs in Plates 2 and 3 brings these responses very forcibly to one's attention.

The red end of the visible spectrum, as noted by earlier investigators under somewhat similar experimental conditions, caused greatly elongated cells which divided infrequently and always in a crosswise direction. Such cell behavior results in an enormously elongated but weak and spindly structure. The blue end of the spectrum, even at low intensities, caused shortened cells which divided more frequently and in a lengthwise as well as crosswise direction. Such cell behavior results in a comparatively short and compact plant body. Daylight, although containing red and blue light, produced effects similar to but not as extreme as those produced by blue light alone. Klebs (1917) asserted that, at high intensities, prothallia grown in red light would develop longitudinal cell walls and eventually plates of cells at the tips of the filaments, but Stephan (1928, 1929), using high intensities, never was able to develop plate-like prothallia in red light. He attributed Klebs' results to failure to use spectroscopically pure screens. The writer was able to get longitudinal divisions in old filamentous prothallia under the G34 screen in bright sunlight, but this screen transmits down to 529 m μ . In moderate intensities, even under G34 glass, cross walls never were formed.

In the light of recent developments relative to plant hormone studies it seems possible that the rôle of radiation in determining the nature of cell growth of fern prothallial cells may be correlated with its effects on hormone activity. It is possible that the red end of the spectrum effects the elaboration of a hormone which provides for the stretching capacity of the cell wall and that the shorter visible radiation of the blue end of the spectrum at least reduces the stretching capacity by its destructive action upon the hormone activity which provides for stretching. Ultra-violet radiation, then, would have a similar destructive effect upon this hormone activity, but to a much more marked degree—roughly, to a degree inversely proportional to the wave-length in its severity—until it would interfere altogether with the life of the cell. Indirect support for this explanation is afforded by the fact that cells, both of seedlings and fern prothallia, which have been subjected to the radiation of the unscreened arc or to that of the arc screened by Corex D glass are extremely brittle. Investigations by Popp and McIlvaine (1937), made subsequent to the completion of this work, indicate that ultra-violet radiation and, to a lesser extent, the blue-violet end of the visible spectrum cause a reduction in the amount of growth substance in turnip seedlings, as determined by the Went method for estimating growth substances.

These findings might explain, also, the difference in rate of reaction caused by the transfer of prothallia from the red to the blue end of the daylight spectrum and vice versa. It will be remembered that lengthwise cell walls appeared in the tip cells of filamentous prothallia in from 36 to 60 hours after the G34 screen had been replaced by the blue screen, while plate-like prothallia developed under the blue screen did not show the beginnings of filamentous outgrowths until more than a week after the blue screen had been replaced by the G34 screen. When the blue screen replaced the G34 screen, the blue end of the spectrum might have destroyed relatively soon the hormone mechanism providing for the stretching of the cell wall and thereby prevented further filamentous development. Under the reverse set of conditions, when the G34 screen replaced the blue one, before any of the relatively isodiametric cells could stretch out and initiate filamentous growth, the hormone mechanism which provides for this stretching capacity had to be elaborated, a process requiring a period of more than a week.

It is interesting to note here Klebs' (1917) rather elaborate explanation of the effect of light quality on prothallia. He thought that at least two photochemical processes were effective in bringing about the characteristic behavior under the red and blue ends of the spectrum, respectively. One, called the *trophische Lichtwirkung*, influences the formation of organic substances, an accumulation of which is necessary for cell division. The other, called the *blastischen Lichtwirkung*, influences the formation of a catalyzer and has a direct influence upon elongation and cell

division, causing elongation under the influence of red light and furthering cell division in blue light. In blue light, therefore, both trophic and blastic influences further cell division; in red light, the two are antagonistic, and the one which predominates determines cell behavior. Klebs' "catalyzer" may be one of the hormones, although his further explanation is probably incorrect.

SUMMARY

Fern prothallia of two species of ferns were grown on either a nutrient solution or on nutrient agar under conditions as uniform as possible to obtain except for differences in the nature of the radiation to which they were subjected. An attempt was made to compare the effects of different ranges of wave lengths upon the growing prothallial cell.

The red end of the visible spectrum of daylight as transmitted by the G34 screen caused excessive elongation of cells developing from spores or of any growing cell of a prothallium of *Osmunda Claytoniana* or *Pteris longifolia*. Cell divisions were relatively infrequent and were solely in a direction at right angles to the lengthwise axis of the cell. Hence a germinating spore developing under such conditions gave rise to a filament of cells altogether unlike the typical heart-shaped prothallium which develops in ordinary daylight. A young growing plate-like prothallium subjected to this kind of radiation sent out filaments of cells and ceased to develop further in its previous manner.

The blue end of the visible spectrum of daylight as transmitted by the blue screen prevented elonga-

tion of cells of developing prothallia. Hence the cells were short and tended to be isodiametric. Cell divisions were relatively frequent and in both crosswise and lengthwise planes of the cell. Hence prothallia developed from germinating spores under these light conditions were plate-like and resembled those developed in white light except for a stubbier appearance. A filamentous prothallium subjected to this type of radiation gave rise to a plate of cells, typically at the apex of the filament. The blue light of the mercury vapor arc also hindered cell elongation.

Long-wave ultra-violet radiation of the mercury vapor arc, as transmitted by the G5S6A screen, had an effect similar to that of blue visible radiation in preventing cell elongation and encouraging cell division.

Except in momentary doses, the shorter ultra-violet wave-lengths of the unscreened mercury arc were lethal. Exposures of a few seconds per day under the conditions specified did not prevent growth altogether, but shortened to a very marked degree filaments which otherwise would have been extremely elongated.

The degree of shortening of cells was roughly proportional to the wave lengths to which the prothallia were subjected, as demonstrated by the results with the mercury lamp in combination with the various screens.

In general, the stunting effect of short-wave radiation on prothallia of *Pteris* and *Osmunda* was found to be somewhat similar to its effect on seed plants, as reported by Popp and Brown.

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STUDIES IN WOOD DECAY. VIII. THE EFFECT OF THE ADDITION OF
DEXTROSE AND DEXTROSE AND ASPARAGINE ON THE RATE
OF DECAY OF NORWAY PINE SAPWOOD BY LENZITES
TRABEA AND LENTINUS LEPIDEUS¹

Henry Schmitz and Frank Kaufert

In a recent paper the authors (Schmitz and Kaufert, 1936) presented experimental evidence to show that the rate of decay of Norway pine sapwood sawdust by *Lenzites trabea* Pers. and of white birch sapwood sawdust by *Polystictus versicolor* Fr. was significantly increased by the addition of asparagine to the sawdust.

Not only because of these significant results but also because of an old belief, now almost universally discarded, that differences in the decay resistance of wood are associated with the presence or absence of readily available carbohydrates and nitrogenous substances, it was felt to be of some interest to determine what effect the addition of dextrose and mixtures of dextrose and asparagine has on the rate of decay of wood by wood-destroying fungi. This paper reports the results of a study to determine the effect of the addition of these substances to Norway pine sapwood on the rate of its decay by *Lenzites trabea* Pers. and *Lentinus lepideus* Fr.

According to Hawley and Wise (1926), the quantity and kinds of sugar formed in wood are not definitely known. If the generally accepted theories of starch and cellulose formation have any factual basis, dextrose must be present in wood. According to these authors, the presence of dextrose in wood is indicated by the seasonal disappearance of the starch which probably results from the enzymatic hydrolysis of starch to maltose and dextrose. The presence of sucrose in the sapwood of several American maples, especially *Acer saccharum*, has been known for a long time. Furthermore, dextrose has been reported in the sap of white birch. The trisaccharide raffinose has been reported present in the twigs of certain conifers.

What precise role the simple sugars in wood play in the nutrition of the wood-destroying fungi is not known.

Gaumann (1927) found that not only the amount of carbohydrates (hexosans) in the stem of spruce and fir fluctuated during the year, but the amount in the heartwood and sapwood of both these tree species fluctuated with a definite periodicity. In spruce sapwood the highest concentration of carbohydrates occurred in March and April, and again in October, and the lowest concentration in February. Although the fluctuations in the concentration of carbohydrates in spruce heartwood were less than in the sapwood, nevertheless a definite periodicity occurred. The highest concentration of carbohydrates in fir sapwood occurred in November and in April.

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Gaumann (1930), however, was unable to find any statistical correlation between the amount of carbohydrates present in these woods at any particular time of the year and their resistance to decay by *Merulius domesticus*, *Polyporus vaporarius*, *Coniophora cerebella*, and *Lenzites abietina*. He therefore concluded that the intensity with which any of these fungi decayed spruce and fir wood was not directly related to the carbohydrate content of the wood.

Sugar solutions of high concentrations have been suggested and have been actually used as a wood preservative and to reduce the swelling and checking of wood. Although little technical and scientific data are available to show the efficacy of such treatments, some general information concerning the use of sugar solutions as a wood preservative is available.

The use of sugar solutions as a wood preservative apparently was proposed first by William Powell of Liverpool, after whom the process is named. In this process the wood is impregnated with a sugar solution consisting principally of molasses and water. Although many claims have been made attesting to the efficacy of this treatment, most of them must be greatly discounted. Nevertheless, some evidence has been presented to show that under certain conditions sugar does increase the serviceability of the wood. For example, Pearson (1929) stated that a diluted sugar solution, to which a small percentage of arsenic is added for protection against the attacks of termites in tropical countries, has been successfully employed in Australia and that extensive tests carried out with Powellized ties on Indian railways gave good results. However, because arsenic is very toxic to many wood-destroying fungi, it is highly probable that the increased decay resistance of the wood is at least partly due to the arsenic.

MATERIALS AND METHODS.—In general, the same methods were used in this study as in the earlier investigation (Schmitz and Kaufert, 1936). Two series of cultures were prepared. To one, dextrose solutions were added; to the other, dextrose and asparagine solutions. In the first series five concentrations of dextrose solution were used: 0.33, 0.66, 1.32, 2.64, and 5.28 per cent. In the second series only three concentrations of dextrose solution were used: 0.33, 1.32, and 5.28 per cent, to which asparagine was also added in either 0.5 or 1.0 per cent concentrations. For each concentration 12 cultures were prepared.

In all cultures 90 cc. of solution were added to 33.0 grams of air-dry Norway pine sapwood sawdust (equivalent to 30.1 grams of oven-dry sawdust).

First the dextrose or dextrose and asparagine solutions were added to the sawdust in the culture jars; next the jars and contents were sterilized in an autoclave for 30 minutes at 10 pounds pressure. After the jars cooled, they were planted with isolates of either *Lenzites trabea* or *Lentinus lepideus* and allowed to grow for three and one half months at about 25°C. At the end of this period the culture jars and contents were dried to constant weight at 104°C. to determine the loss in weight from decay.

RESULTS AND DISCUSSION.—*Loss in weight percentages based on the oven-dry weight of sawdust without the weight of nutrients added.*—The effect of adding various amounts of dextrose and a mixture of dextrose and asparagine to Norway pine sapwood on its rate of decay by *Lenzites trabea* and *Lentinus lepideus* is shown in table 1.

TABLE 1. *The effect of adding dextrose and a mixture of dextrose and asparagine to Norway pine sapwood sawdust on its rate of decay by Lenzites trabea and Lentinus lepideus. (Weight of sugar and asparagine disregarded in computing percentage losses.)*

Concentration of dextrose in the water added	Concentration of asparagine in the water added	Loss in weight ^a	
		<i>Lenzites trabea</i>	<i>Lentinus lepideus</i>
Per cent	Per cent	Per cent	Per cent
0.0	0.0	36.5 ± 1.41	20.4 ± 0.37
0.33	0.0	46.1 ± 1.54	17.9 ± 0.50
0.66	0.0	43.7 ± 1.69	17.2 ± 0.49
1.32	0.0	37.7 ± 1.14	14.9 ± 0.41
2.64	0.0	40.5 ± 1.62	9.5 ± 0.51
5.28	0.0	38.0 ± 1.70	0.5 ± 0.21
0.33	0.5	46.4 ± 3.82	5.3 ± 1.00
1.32	0.5	43.6 ± 3.11	4.8 ± 0.31
5.32	0.5	32.8 ± 5.15	2.1 ± 0.38
0.33	1.0	30.9 ± 2.60	3.6 ± 0.25
1.32	1.0	26.2 ± 2.20	3.4 ± 0.32
5.28	1.0	13.7 ± 1.24	b 2.2 ± 0.34

^a The standard errors are not expressed as percentages of the mean percentage loss in weight.

^b Increase.

The average losses in weight based on the oven-dry weight of sawdust alone and their standard errors are given in table 1. The significance of the differences between the mean of the control cultures, each mean of the culture receiving dextrose and mixtures of dextrose and asparagine, was determined by using the standard error of a difference formula and the *t* test.

The weight of dextrose and dextrose and asparagine was not included in calculating the losses in weight given in table 1. Although this introduces only small errors in the series to which small amounts of dextrose or dextrose and asparagine were added, it introduces rather large errors in the series to which large amounts of dextrose and asparagine were added. Be-

cause the flasks had been discarded before this source of error was discovered, no systematic study was made of the amount of sugar remaining in the cultures after the incubation period. It was assumed that the fungi would rapidly utilize the small amounts of dextrose added to some series and probably would utilize rapidly even the large amounts added to the other series. However, qualitative tests in a somewhat similar series of cultures showed the presence of large quantities of sugar at the end of the incubation period, especially in those cultures to which large amounts of dextrose had been added.

If all the observed loss in weight is assumed for the moment to be the result of the breaking down of the wood, this of course implies that the dextrose and dextrose and asparagine added were either first completely or concomitantly utilized by the fungus. If this hypothesis be true, then certain tentative conclusions may be drawn. Obviously, in those cultures where the losses in weight were greater than the loss in weight of the control, the error introduced by the weight of dextrose or dextrose and asparagine should affect but little the validity of the observed results. Only where the observed loss in weight is less than that of the control are the observed results of doubtful validity.

The addition of small quantities of dextrose appears to cause statistically significant increases in the rate of decay of Norway pine sapwood sawdust by *Lenzites trabea*. The addition of larger quantities, however, appeared to neither significantly increase nor significantly decrease the rate of decay by this fungus. The losses in weight of the cultures to which 1.32, 2.64, and 5.28 per cent dextrose solution was added, respectively, lost about the same weight as the controls.

The addition of even small amounts of dextrose causes a statistically significant decrease in the rate of decay of Norway pine sapwood by *Lentinus lepideus*. Although the relationship was not tested for statistical significance, it appears that as the amount of dextrose is increased, the rate of decay of Norway pine sapwood is decreased.

The effect of dextrose solutions plus 0.5 per cent asparagine on the rate of decay of Norway pine sapwood by *Lenzites trabea* is somewhat similar to that caused by dextrose alone. The addition of either a 0.33 or a 1.32 per cent dextrose solution containing 0.5 per cent asparagine significantly increased the rate of decay by *Lenzites trabea*. A higher concentration of dextrose containing 0.5 per cent asparagine did not cause a significant increase or decrease in the rate of decay.

The effects of dextrose solutions containing 1.0 per cent asparagine on the rate of decay by *Lenzites trabea* were somewhat different from those caused by the addition of similar concentrations of dextrose containing 0.5 per cent asparagine. In every series the addition of dextrose solutions containing 1.0 per cent asparagine caused a decrease in the rate of decay of Norway pine sapwood by *Lenzites trabea*. These

TABLE 2. The effect of adding dextrose and a mixture of dextrose and asparagine to Norway pine sapwood on its rate of decay by *Lenzites trabea* and *Lentinus lepideus*. (Weight of sugar and asparagine added to weight of sawdust.)

Oven dry weight of sawdust, grams	Weight of dextrose, grams	Weight of asparagine, grams	Total weight of dextrose, sawdust and asparagine, grams	<i>Lenzites trabea</i>	<i>Lentinus lepideus</i>
Control					
30.1	0.0	0.0	30.1	36.5	20.3
Glucose Series					
30.1	0.3	0.0	30.4	46.6 ^b	18.7 ^b
30.1	0.6	0.0	30.7	44.8 ^b	18.8 ^b
30.1	1.2	0.0	31.3	40.1 ^b	18.1 ^b
30.1	2.4	0.0	32.5	44.9 ^b	16.1 ^b
30.1	4.8	0.0	34.9	46.6 ^b	14.2 ^b
Glucose plus asparagine series					
30.1	0.3	0.45	30.85	47.3 ^b	7.6 ^b
30.1	0.3	0.9	31.3	33.4	7.3 ^b
30.1	1.2	0.45	31.75	45.7 ^b	9.7 ^b
30.1	1.2	0.90	32.2	31.0	9.7 ^b
30.1	4.8	0.45	35.35	43.5	16.7 ^b
30.1	4.8	0.90	35.3	27.5 ^b	— ^a

^a This value was omitted from the analysis of variance because of the large number of missing values in the series.

^b These figures are significantly different from control.

decreases are statistically significant except in the case of the solution containing 0.33 per cent dextrose plus 1.0 per cent asparagine.

The addition of dextrose and asparagine to Norway pine sapwood resulted in a pronounced decrease in its rate of decay by *Lentinus lepideus*. Whether the dextrose solutions contained 0.5 or 1.0 per cent asparagine apparently made little difference. Every concentration of dextrose and asparagine resulted in a significant decrease in the rate of decay of Norway pine sapwood sawdust by *Lentinus lepideus*.

Loss-in-weight percentages based on the oven-dry weight of sawdust plus the weight of dextrose and asparagine.—When the loss-in-weight percentages given in table 1 were computed, the weights of dextrose and of dextrose and asparagine were not included in the oven-dry weight of the sawdust; but in calculating the percentages in table 2, the weight of dextrose and the weight of dextrose and asparagine were included. These loss-in-weight percentages based on the oven-dry weight plus the nutrients, therefore, might be considered an indication of the total metabolic activity of the fungus that resulted in the breakdown of both the wood and the dextrose and asparagine. Obviously, the actual amount of wood and nutrients utilized by the fungi is not known.

The significance of the differences of the percentage losses in weight based on the weight of sawdust plus the nutrients was determined by Fisher's analysis of variance method.²

The analysis of variance showed that the mean percentage loss in total weight by *Lenzites trabea* was significantly increased when dextrose alone was

added to Norway pine sapwood sawdust. In fact, all the differences were highly significant except the difference between the control and the mean of the cultures to which 1.2 grams of dextrose were added.

On the other hand, the mean percentage loss in total weight by *Lentinus lepideus*, when only dextrose was added to sawdust from Norway pine sapwood, was significantly decreased. For this fungus the losses for all the concentrations of dextrose were significantly smaller than the mean loss for the control.

When both dextrose and asparagine were added to Norway pine sapwood, the percentage loss of total weight by *Lenzites trabea*, on the average, was significantly different from the control mean loss; but not all the individual differences were significantly different or consistently larger or smaller. The addition of 0.3 gram dextrose plus 0.45 gram asparagine, and also 1.2 grams dextrose plus 0.45 gram of asparagine, resulted in a significantly greater percentage of total weight. The addition of 0.9 gram asparagine plus 4.8 grams of dextrose caused a significant decrease in the percentage loss of the total weight. The mean losses for the other combinations of dextrose and asparagine were not significantly different from the mean loss of the controls.

The addition of dextrose and asparagine to sawdust from Norway pine sapwood inoculated with *Lentinus lepideus* resulted in significantly different mean losses. For all combinations the differences between the control and the treated means are highly significant.

These data and the results of their analysis indicate rather clearly that the addition of small or even of considerable quantities of dextrose increased the rate of decay of sawdust from Norway pine sapwood by *Lenzites trabea*. The rate of decay of sawdust from Norway pine sapwood by *Lentinus lepideus*, on

² The writers are greatly indebted to Prof. R. M. Brown, Division of Forestry, University of Minnesota, for making this analysis.

the other hand, was decreased slightly when comparatively small amounts of dextrose were added to the culture and was greatly decreased when larger amounts of dextrose were added.

The rate of decay of Norway pine sapwood sawdust by *Lenzites trabea* was also increased by the addition of small amounts of dextrose and asparagine. However, if the amounts of the substances, especially of asparagine, were increased, the rate of decay by this fungus was decreased. The addition of both dextrose and asparagine caused an even greater decrease in the rate of decay by *Lentinus lepideus* than when dextrose alone was added.

The nutrient agar was prepared as follows: 50 grams of air-dried sawdust from Norway pine sapwood and 1000 cc. of distilled water was digested in the autoclave for one-half hour at 15 pounds pressure. After the liquid had cooled, it was decanted from the sawdust and then filtered. This filtrate was used to make up nutrient agar containing $1\frac{1}{2}$ per cent agar. To this filtrate either the following concentrations of dextrose or a mixture of dextrose and asparagine were added: (1) Control, no dextrose or asparagine added; (2) dextrose 0.33 per cent; (3) dextrose 0.33 per cent, asparagine 0.5 per cent; (4) dextrose 0.33 per cent, asparagine 1.0 per cent; (5) dextrose 0.66 per cent;

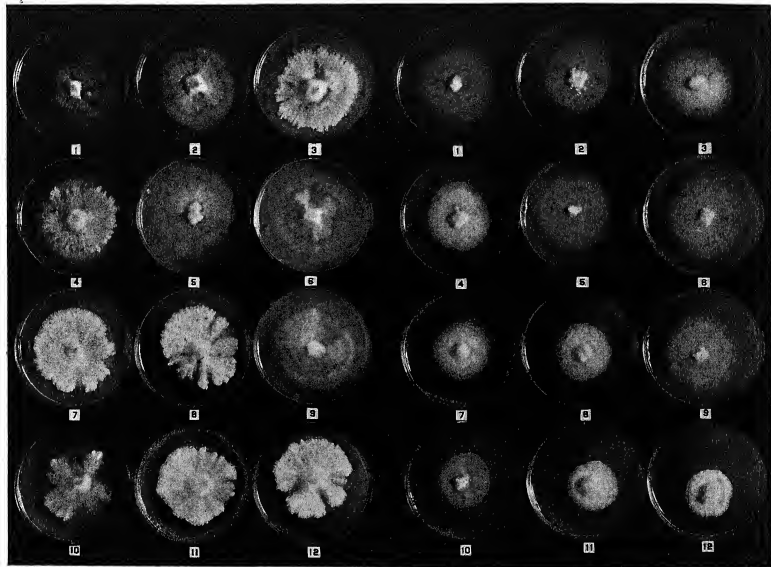


Fig. 1. The radial growth and appearance of colonies of *Lenzites trabea* and *Lentinus lepideus* after 8 days on Norway pine sapwood extract-agar containing various amounts of dextrose and asparagine. *Lenzites trabea* (left), *Lentinus lepideus* (right).

1. Control, no dextrose or asparagine added; 2. Dextrose 0.33 per cent; 3. Dextrose 0.33 per cent, asparagine 0.5 per cent; 4. Dextrose 0.33 per cent, asparagine 0.1 per cent; 5. Dextrose 0.66 per cent; 6. Dextrose 1.32 per cent; 7. Dextrose 1.32 per cent, asparagine 0.5 per cent; 8. Dextrose 1.32 per cent, asparagine 1.0 per cent; 9. Dextrose 2.65 per cent; 10. Dextrose 5.28 per cent; 11. Dextrose 5.28 per cent, asparagine 0.5 per cent; 12. Dextrose 5.28 per cent, asparagine 1.0 per cent.

The question naturally arises why the addition of dextrose and especially of dextrose and asparagine should, in some instances at least, adversely affect the rate of decay of wood by wood-destroying fungi. In order to throw some additional light on this question, the growth of the two fungi on Norway pine sapwood extract-agar containing various concentrations of dextrose and asparagine was studied.

(6) dextrose 1.32 per cent; (7) dextrose 1.32 per cent, asparagine 0.5 per cent; (8) dextrose 1.32 per cent, asparagine 1.0 per cent; (9) dextrose 2.64 per cent; (10) dextrose 5.28 per cent; (11) dextrose 5.28 per cent, asparagine 0.5 per cent; (12) dextrose 5.28 per cent, asparagine 1.0 per cent.

The nutrient agar was sterilized and poured into Petri dishes. Four dishes were prepared for each



Fig. 2. The amount of mycelial growth of *Lentinus lepideus* on Norway pine sawdust containing dextrose and dextrose and asparagine. The amount of mycelium in Jar 5, for example, to which 5.28 per cent dextrose was added is far greater than that in Jar 12, the control, to which no dextrose or asparagine had been added. Nevertheless, Jar 5 lost much less weight than Jar 12.

1. 90 cc. of 0.33 per cent dextrose solution added; 2. 90 cc. of 0.66 per cent dextrose solution added; 3. 90 cc. of 1.32 per cent dextrose solution added; 4. 90 cc. of 2.66 per cent dextrose solution added; 5. 90 cc. of 5.28 per cent dextrose solution added; 6. 90 cc. of 0.33 per cent dextrose, 0.5 per cent asparagine solution added; 7. 90 cc. of 1.32 per cent dextrose, 0.5 per cent asparagine solution added; 8. 90 cc. of 5.28 per cent dextrose, 0.5 per cent asparagine solution added; 9. 90 cc. of 0.33 per cent dextrose, 1.0 per cent asparagine solution added; 10. 90 cc. of 1.32 per cent dextrose, 1.0 per cent asparagine solution added; 11. 90 cc. of 5.28 per cent dextrose, 1.0 per cent asparagine solution added; 12. 90 cc. of distilled water added.

concentration, two of which were planted with isolates of *Lenzites trabea* and two with isolates of *Lentinus lepideus*, and then incubated at 28°C. From time to time the average radial growth of each fungal colony was measured and recorded. It is recognized that colony size is not an index of the amount of growth of a fungus. A small colony may produce more mycelium than a large colony. This is indicated in figure 1.

The radial growth of *Lenzites trabea* on agar was increased somewhat by the presence of dextrose except for the highest concentration. Although the addition of a mixture of asparagine and dextrose does not greatly influence the radial growth of *Lenzites trabea*, it does change materially the character of the fungal growth. In all cultures to which asparagine was added, the mycelial mat was much more copious

and fluffy than the mycelial mat of the control and dextrose cultures. In these, especially the controls, it was appressed closely to the surface of the nutrient agar.

The presence of sugar in the culture medium in concentrations up to 2.64 per cent had little effect on the radial growth of *Lentinus lepideus* on agar. On the other hand, the presence of 5.28 per cent of sugar considerably retarded its radial growth. The presence of asparagine either in 0.5 or 1.0 per cent concentrations in every case not only retarded the radial growth of *Lentinus lepideus*, but also materially influenced the character of the mycelial mat. The presence on the agar plates of pieces of agar from the stock cultures is objectionable in this work. If the agar plates had been greatly deficient in nutrients, the presence of pieces of agar from the stock

cultures might have been the source of considerable error. However, under the conditions of the experiment the influence of the presence of the stock culture agar on the plates is believed to have had relatively little influence on the observed results.

In general, the results obtained from both the Petri dish cultures and the sawdust cultures corresponded rather closely. This similarity may suggest a possible explanation for not only the extensive growth of mycelium in the sawdust cultures containing dextrose and asparagine, but also their small loss in weight.

The addition to the sawdust cultures of dextrose, either alone or in combination with asparagine, greatly increased the amount of mycelium. In the dextrose series inoculated with *Lentinus lepideus*, for example, the amount increased with an increase of dextrose. For this reason the cultures to which 5.28 per cent dextrose had been added would have been given first place and the controls last place, if an estimate of the expected loss in weight had been based on the volume of mycelium produced. Actually, the 5.28 per cent dextrose cultures lost considerably less weight than the controls. The amount of fungal growth in the *Lentinus lepideus* cultures to which dextrose or dextrose and asparagine was added is shown in figure 2.

This observed relationship between the volume of mycelium produced and the presence of dextrose or dextrose and asparagine, may justify the following tentative hypothesis: that the test fungi, *Lenzites trabea* and *Lentinus lepideus*, differ markedly with respect to their ability to attack Norway pine sawdust when more available nutrients, such as dextrose alone or both dextrose and asparagine, are present. *Lenzites trabea* does not appear to exhibit selective feeding, the wood substance appears to be destroyed at the same or somewhat increased rates when the more available nutrients are present. *Lentinus lepideus*, on the other hand, appears to utilize the more available nutrients and produces large quantities of vegetative mycelium but does not attack the

wood substance as rapidly in the presence of nutrients as it does when these are absent. The stimulation of mycelial growth of *Lentinus lepideus* by concentrations of dextrose and dextrose and asparagine that reduced its ability to decay wood would appear to largely exclude the possibility that the observed retardation may have been due to osmotic effects.

SUMMARY

A study of the effect of the addition to wood of dextrose and dextrose and asparagine on its rate of decay by wood-destroying fungi indicates that the rate of decay of wood caused by some fungi, *Lenzites trabea* for example, may be increased by the addition of dextrose or dextrose and asparagine if the amounts added are not too great. In the case of other fungi, *Lentinus lepideus* for example, it appears that the rate of decay of wood it causes is decreased if dextrose or dextrose and asparagine is added to the culture. In such cases the fungus may for a time at least develop largely at the expense of the sugar and asparagine present and only to a limited degree at the expense of the wood substance.

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THE AVAILABILITY OF DIFFERENT FORMS OF NITROGEN TO A GREEN ALGA¹

C. A. Ludwig

CONSIDERABLE WORK has been done on the utilization of different compounds as sources of nitrogen for green plants. Much of this was with higher plants, sometimes under conditions which assured neither sterility nor even a low plane of microbiological activity in the medium surrounding the roots. Under such conditions there could be no certainty when assimilation occurred that the nitrogenous compounds supplied were the ones actually absorbed by

the plants. Some work has also been done with algae. Part of it is subject to the same criticism, but much has been under strict biological control, which is easier with microscopic plants. In practically all cases the criterion of nitrogen absorption was the qualitative one of the appearance of visible growth. The work reported here, conducted in connection with investigations in this laboratory on biological nitrogen fixation, amplifies the previous work on algae with quantitative data for the assimilation of nitrogen from several compounds by a unicellular organism in pure culture.

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The writer is indebted to Ellen K. Rist, of this laboratory, for most of the nitrogen determinations made during this study.

The literature on the subject of nitrogen absorption by higher plants has been reviewed (3, 19, 42, 77a, 79). While this has not been done recently for the algae, it is not attempted here except with regard to the particular compounds tested. No papers reporting assimilation are cited except where it seems fairly certain that the cultures were genuine pure cultures and that the substance under study was the only source of nitrogen available to the organism. (But in general the possibility of nitrogenous impurities in the reagents or of changes during heat sterilization is not considered.) On the other hand, in instances where non-assimilation or toxicity was reported, it has been considered that moderate contaminations with other organisms, or the presence of other nitrogen sources in the medium, did not contribute much doubt as to the significance of the results; and a number of such studies are cited.

METHODS.—The organism used was a species of *Chlorella* (Dr. F. B. Wann's No. 11, obtained from Dr. Franklin E. Allison, of this laboratory). The advantage of a unicellular plant for work of this type lies in the fact that cultures can be grown by the usual bacteriological methods, thus simplifying the problem of sterility.

The solution for the control cultures contained nitrogen as potassium nitrate and had approximately the following composition: $MgSO_4$, 0.01 M.; KNO_3 , 0.0125 M.; KH_2PO_4 , 0.009 M.; $Fe_2(SO_4)_3$, 0.0000106 M.

For each experimental culture, except as noted, the potassium nitrate was replaced with the equivalent amount (to supply approximately 175 mg. nitrogen per liter) of the nitrogenous compound to be tested. The chemicals were usually ordinary "C.P." materials, while the magnesium sulphate was a therapeutic preparation of Epsom salts. The ammonium acetate, citrate, tartrate, formate, succinate, and lactate were prepared by neutralizing the acids with excess of strong ammonia and boiling off the excess. In a few cases the salt was concentrated under reduced pressure, crystallized, and washed; but more often it was used directly without purification. The stock solutions were analyzed for ammonia by distillation with sodium hydroxide and were found to contain approximately the expected amounts. Tap water was used except in a few experiments where parallel cultures were grown in media made up with distilled water. These cultures showed no evident advantages for the distilled water media, and tap water was then used exclusively on account of the greater certainty of including sufficient traces of those necessary elements which were not added specifically. Cultures in which no nitrogen was added were always prepared. They always grew for a few days, but soon became yellow, and on analysis showed not more than a trace of nitrogen, the value of which was used as a control correction.

The pH was measured colorimetrically, but was not controlled closely. It most often had some value between 5 and 7 after sterilization of the medium and

before inoculation. The pH at this time is probably of little importance, however, unless it is fairly acid, because determinations at the close of an experiment, when taken promptly, showed a preponderance of values in the neighborhood of 5.5 or lower. This fact suggests that the carbon dioxide (5 per cent) in the aeration stream had fixed the reaction approximately at pH 5.5 in most of the cultures which were not for some reason more acid. This reaction is one at which the organism² has been shown by the work of Hopkins and Wann (41b, 41c, 101a) to grow well and at which the amount of available iron is not likely to be limiting. In flasks where calcium carbonate ("precipitated chalk" 1 gm. or ground marble 2 gm. per liter) was used, the pH was probably higher directly around the cells, since they always settled to the bottom in close contact with the material. The growth results render it doubtful, however, that there was any significant removal of iron from the solution by the calcium carbonate such as the authors just cited (41b, 41c, 41d, 101a) observed when calcium phosphate was precipitated in the solution.

Media were sterilized in the autoclave except as indicated otherwise. No tests were made to determine if any decomposition of the nitrogenous materials occurred during this treatment. However, most of the compounds are fairly stable at the temperature concerned, and with the exception of the non-assimilable materials and of urea, the amount of nitrogen absorbed by the organisms was greater than that which it seems at all likely would be transformed to some other form during sterilization. Inoculations with equal volumes of a well shaken suspension of the organism were made with a sterile pipette. Contaminations were rare. Where they occurred, the cultures were either rejected or the presence of the foreign organism was considered in interpreting the results.

The culture vessels were pyrex Erlenmeyer flasks closed with 2-hole rubber stoppers fitted with glass tubes for aeration. In the first experiment one liter, in the second 100 ml., and in all later ones 200 ml. of solution per culture was used. All cultures were run in duplicate in each experiment, and the results given are means of the two cultures unless otherwise indicated. In general duplicates agreed well, although a twofold variation was not very unusual, and a greater occurred occasionally.

The aeration stream consisted of air enriched with approximately 5 per cent of carbon dioxide. It was bubbled through strong sulphuric acid, mercuric chloride solution, and then three to five (usually four) of the culture flasks in series at a rate which allowed apparently undiminished growth in the last flask.

Light was furnished by mazda lamps of clear glass with porcelain reflectors. The intensity varied in the different experiments but was around 300–500 foot candles (3200–5400 lumens). Illumination was

² While the organism used by these authors is designated merely as *Chlorella* sp., Dr. Hopkins stated in a personal communication to the writer that the one used in all their work together was *Chlorella* No. 11.

continuous except in the last experiment, where the lighted and unlighted periods were 16 and 8 hours, respectively. In the second experiment part and in later experiments all of the culture flasks were placed in a white tray containing a layer of water about as deep as that of the culture solution.

At the close of an experiment the cells in each culture were collected on filter paper, washed with distilled water, dried, and the nitrogen determined by the Kjeldahl method, using copper sulphate as the catalyst in the earlier work and elemental selenium in the later.

RESULTS.—The results are summarized in table 1. The mean uptake of nitrogen per culture is given for each material, both as mg. per culture and as a percentage of the amount absorbed by the corresponding (potassium nitrate) control cultures. Theoretically it should be possible, by comparing the means of the percentages, to list all the different nitrogen sources in the order of their utilization. However, some of the means are from only a few determinations where the variability was large, thus making large differences necessary for significance. And even if this were not true, it would hardly be justifiable to carry the comparison so far. A number of investigators (26, 29, 81, 95) have called attention to the fact that many plants absorb nitrate nitrogen best at a more acid reaction than that at which they use ammonium nitrogen best. Urhan (100) has shown that some species of *Chlorella* and *Scenedesmus* take up nitrogen from nitrate and nitrite best in a moderately acid medium and from ammonium in a more alkaline one. Tiedjens (95) has further made the point that where the nutritive values of different nitrogen carriers are being compared, the conditions for each should be optimum for its assimilation. Since it was impractical to determine and employ such conditions for each nitrogen source considered, small differences must be considered as without significance. In many cases, however, differences are great enough to be fully significant or at least highly suggestive; and in practically all cases the results are ample for answering the question primarily under investigation—Is the nitrogen in the compound assimilable?

It will be seen from table 1 that the organism absorbed nitrogen from a very considerable number of different nitrogen sources, both inorganic and organic, but did not do so from others, some of which might have been expected to be suitable sources of the element.

Discussion.—*Potassium nitrate.*—The growth, as shown both by the appearance of the cultures and by the amount of nitrogen assimilated, was good. This was to be expected, since almost all reported tests of algae with nitrate in the past have shown its utilization. But most pure cultures have been isolated with nitrate-containing media, and it is possible that the use of a different source of nitrogen in the isolation medium might give organisms of a different character. Since the general situation is so well known, and the number of studies which could be mentioned is very

large, none will be cited in this connection. Nitrate is not assimilable by all algae, however. Some of the flagellated forms, especially those of the genus *Euglena*,³ are notable as being unable to use this material or to use it only with difficulty (31, 32, 34, 35, 66). However, some other algae seem also to be lacking in this ability (13, 25, 51, 88), although it is possible that most of them would show the ability if the proper experimental conditions were provided. Thus, doubt as to the reported result for *Scenedesmus acutus* Meyen (13) has been expressed by Klebs (32, p. 183) and Senn (89, p. 71); and Grintzesco (39) has reported a definitely contrary finding.

Potassium nitrite.—The nitrite was assimilable in the low concentrations used, but even these concentrations seemed slightly toxic when added to a nitrate medium. Possibly a more alkaline medium would have given better results with nitrite. The result agrees with that of most other work with algae on this point, for while there is abundant evidence (8, 9, 12, 14, 17, 18, 27, 47, 72, 77, 83, 98, 100, 102) that nitrite is assimilable under proper conditions, it is also common experience that it is non-assimilable or toxic in too high concentration (10, 17, 72, 75). In view of the general acceptance of the idea that nitrite is an intermediate stage in the reduction of nitrate by higher green plants, it is interesting to note that Beckwith (11, 12), Hall (41a), ZoBell (102), and Sommer (90) have recently produced evidence that the same is true for unicellular green algae. Warburg and Negelein (101b) have shown that *Chlorella pyrenoida* Chick may produce nitrite from nitrate, but the result was observed only at high acidity in the dark and probably means little where the normal metabolism of the organism is concerned.

Inorganic ammonium salts.—It is convenient to discuss together the assimilation of nitrogen from ammonium sulphate, ammonium chloride, ammonium phosphate, and ammonium carbonate since the amounts of nitrogen taken up from these sources were nearly the same. These amounts were somewhat greater than those secured from the control solutions, and this fact suggests a superiority of ammonium over nitrate as a source of nitrogen under the conditions of the experiments. Moreover reduction to about 3.5 in the pH of the sulphate and chloride media during growth, a greater nitrogen absorption and smaller pH change where nitrate and ammonium were used in combination, and the result from adding calcium carbonate to the phosphate medium suggest in addition that with better buffering the superiority might have been even greater.⁴ Results with these nutrients by other in-

³ It is realized that the *Euglenae* and similar organisms are probably animals rather than plants, but many of them contain chlorophyll and exhibit a metabolism essentially like that of green plants. Their nitrogen nutrition is therefore interesting in connection with this study and citations concerning it are included.

⁴ These remarks refer to nitrogen uptake, only. Dry matter production might give a somewhat different result, since assimilated nitrate nitrogen is often more

investigators have usually been essentially the same as reported here. Thus ammonium sulphate (2, 4, 7, 9, 20, 21, 22, 28, 31, 34, 35, 38, 50, 60, 66, 71, 72, 73, 74, 75, 76, 77, 83, 84, 88, 94, 99, 101, 102), ammonium chloride (17, 18, 23, 25, 36, 47, 54, 55, 60, 85a, 88, 102), and ammonium phosphate (31, 34, 35, 38, 56, 66, 71, 72, 82, 83, 84, 94, 102) have all been found to be suitable sources of nitrogen to various species of algae under the proper conditions. On the other hand, to occasional species some of these inorganic ammonium salts have occasionally proved to be unavailable or even toxic (10, 13, 25, 28, 32, 35, 51, 62, 88, 92, 96). In general it appears that algae which cannot use inorganic sources of nitrogen, or use them with difficulty, usually occur in nature in habitats such as sewage, contaminated water, heavily manured soils, etc., which contain a great deal of soluble organic nitrogen.

Because of the volatility of $(\text{NH}_4)_2\text{CO}_3$, a carbonate solution was sterilized separately and analyzed for ammonia, after which the proper amount was added aseptically to the culture solution. The pH was usually considerably above 7.0 before inoculation (but see above). Considerable ammonium carbonate probably was present also in the ammonium oxalate plus calcium carbonate medium because of the very low solubility of calcium oxalate, and in the urea medium because of conversion of the urea during sterilization. Nitrogen was well utilized from all these solutions. In the few previous studies found (12, 49, 82, 94, 102) ammonium carbonate was reported to support growth of algae. To these should perhaps be added those reporting assimilation of urea, for which see below. It is likewise possible that the non-availability or deleterious effects sometimes reported for urea should be credited to this compound instead.

Organic ammonium salts.—It will be convenient to discuss these compounds in two groups, the first to include the lactate, oxalate, succinate, tartrate, and citrate and the second the salts of the first three members of the fatty acid series—formate, acetate, and propionate. Reference to table 1 shows that the organism assimilated more nitrogen from each of the compounds of the first group than from nitrate or from any of the inorganic ammonium salts used. This may be due to secondary rather than to direct effects of the anions, for a medium with an organic ammonium salt will develop less acidity for a given uptake of nitrogen than will one with a salt of an inorganic and therefore stronger acid. Also there is the possibility that the organic acid radical can be used as a supplementary source of carbon or that it increases the availability of the iron. The good results with the oxalate are especially interesting in view of the fact that oxalate is very toxic to many plants. Since oxalate toxicity is apparently due in most cases to the precipitation of calcium, its absence in this instance is to be considered as verification of Hopkins and efficient than ammonia nitrogen in the synthesis of plant substance, but the lack of dry weights renders such a comparison impossible here.

Wann's finding (41b) that the organism requires little or none of this element.

The results show also that under the conditions involved, ammonium ion is more readily absorbed than nitrate. In this connection it is interesting to note that Pearsall and Loose (80) have recently reported that *Chlorella vulgaris* removed ammonia more rapidly than nitrate from a medium containing NH_4NO_3 , while Braarud and Føyn (18) reported that a species of *Chlamydomonas* removed nitrate and nitrite more rapidly than ammonia from a medium containing NaNO_3 , NaNO_2 , and NH_4Cl .

Not much previous work has been done on the assimilation of nitrogen from these salts by algae. Ammonium lactate (99), citrate (99, 52), and tartrate (6, 9, 25, 50, 88) have been reported to be utilizable by algae. However, the tartrate (48, 88) and the lactate, succinate, and citrate (35) have also been reported to be unavailable to some organisms. In fact, the addition of a citrate to a peptone medium (35) prevented the development of *Euglena deses* Ehrenb. Ammonium oxalate (53) in an otherwise suitable medium prevented the development of *Porphyridium cruentum* Naeg., but calcium oxalate was very favorable to it.

For the ammonium salts of the lower fatty acids the results show a lower assimilation of nitrogen from the formate than from the control, still less from the acetate, and none at all from the propionate. The acetate was not toxic when used in half the usual concentration in a medium containing available nitrogen from another source but was unavailable or practically so at that and higher concentrations when used alone. The half concentration of propionate prevented utilization of nitrogen which was otherwise available. It does not seem likely that the failure of the acetate or propionate to support growth was due to impurities in the preparations. It cannot have been due to the ammonia, for in the first acetate experiment the ammonia was taken from the same bottle as used for other preparations which were well utilized. In later ones the salt was crystallized and washed before being used. This later preparation supported growth in low concentration and did not prevent growth on other nitrogen sources in half concentration. Also, medium for part of the cultures with this preparation was sterilized by filtration through porous porcelain. The medium sterilized by this means did not support growth at the usual concentration, thus indicating that the manner of sterilization did not alter the results obtained. The ammonium propionate was prepared from freshly distilled acid and was crystallized, washed, and dried before use.

It is interesting to note that some previous workers have found salts of the lower fatty acids to be harmful to the organisms they used, while others have found them harmless or even valuable as a source of carbon. Kufferath (53) found that both sodium formate and calcium acetate prevented the growth of *Porphyridium cruentum* Naeg. on an agar medium, Dusi (35) found that ammonium acetate would not

support growth of *Euglena anabaena* var. *minor* Mainx, and Jacobsen (45) reported a similar effect of these salts on *Haematococcus pluvialis* at a concentration of one per cent in a medium containing NH_4NO_3 as a nitrogen source. On the other hand, Pringsheim (85) found that *Chlorogonium euchlorum* could be grown in the dark with ammonium acetate, and has called attention to the fact that, for continued thriving in the dark on a medium containing highly hydrolyzed muscle protein as a nitrogen source, *E. gracilis* requires in addition a fatty acid—acetic, butyric, or caproic, preferably—as a carbon source. Acids much higher in the series, other organic acids, and sugars are not suitable. Other investigators (9, 30, 37, 58, 99) have reported work showing that some algae, often other than the Chlamydomonadaceae, can endure and often utilize the acetate ion. In some of these cases (9, 99) the organism involved grew with ammonium acetate as the sole source of nitrogen.

The progressive unavailability to the present organism of the nitrogen in these fatty acid salts is puzzling in view of its great availability in the other organic acid salts studied. The results are somewhat suggestive of a progressive toxicity of the anions but unfortunately no positive statement can be made, as it was impossible to continue the work till this point could be determined. The use of calcium chloride, magnesium chloride, and aluminum chloride in one experiment was to test the possibility that the effect is capable of prevention by an application of the principle of the antagonism of ions.⁵ The results indicate that neither calcium, magnesium, nor aluminum ions antagonize those of either of the three acids under the conditions involved. In fact, in the only case (formate) in which growth occurred at the usual concentration, it was either unaffected or poorer in the presence of the extra salt.

Acetamide.—Acetamide was found to be a good source of nitrogen for the organism. This result is in line with some previous reports (9, 25, 65, 83, 85a) but apparently contrary to some others, in one of which (83) it is claimed that certain blue-green algae cannot assimilate acetamide, and in another of which (50) two species of flagellates are said to be unable to assimilate acetamide, propionamide, butyramide, or valeramide.

Urea.—The urea medium was found to contain a ready source of nitrogen. Other investigators (8, 11, 12, 17, 18, 23, 65) have also reported similar results. It is possible, however, with all these results that enough conversion of the urea to ammonium carbonate had taken place during sterilization to render the results questionable. Thus Chick (23) found that her urea-containing control liquid yielded about 25 per cent of its nitrogen as free ammonia. In still other experiments the material (or its breakdown products) has been found unavailable or harmful (52, 62). The value of this substance needs further study

under conditions which will insure that it alone is presented to the plant as a nitrogen source.

Uric acid.—This compound proved to be an excellent nitrogen source in spite of considerable acidity of the medium, this result being in agreement with most other findings (23, 84, 94). In one published result (8), however, the material was found to be almost, and perhaps absolutely, unavailable.

Guanidine carbonate.—This compound was tested in several concentrations and was found to be an excellent source of nitrogen in all of them. Loew and Bokorny (62) reported guanidine toxic to species of *Spirogyra* but thought the toxicity might be due to the alkalinity of the solution.

Hydroxylamine and glucosamine.—Hydroxylamine and glucosamine were used as hydrochlorides. The failure to grow in the first of the two experiments could have been due to the high acidity, pH 3 to 4; but in the second experiment the reaction was corrected by adding calcium carbonate. Few workers have reported on these compounds, but Jacobsen (44) claimed that *Chlorogonium euchlorum* Ehrh. utilized glucosamine, while Lutz (64) and Loew (61) have both reported hydroxylamine as toxic. Usami (100a) reported M/1000 hydroxylamine hydrochloride as toxic to *Fontinalis antipyretica* L., a bryophyte.

Amino acids.—The compounds to be considered under this heading are glycine, alanine, valine, leucine, phenylalanine, tyrosine, l-cystine, aspartic acid, glutamic acid, and for convenience, asparagine. With the exception of aspartic and glutamic acids used alone, when they produced a pH around 3 in the media, these substances all supported growth to some degree, and some, including glycine, alanine, asparagine, and aspartic and glutamic acids when neutralized with calcium carbonate, supported heavy growth and permitted a large accumulation of nitrogen in the cells. No previous work seems to have been done to determine the availability to algae of cystine, but varying results have been reported for the others. Thus a good many workers, using altogether a considerable number of different species of algae, have found that glycine (7, 9, 16, 18, 25, 27, 28, 33, 34, 35, 43, 47, 57, 66, 77, 83, 85a, 94, 96, 101), alanine (9, 16, 18, 25, 33, 34, 35, 94, 96), valine (33, 34, 35, 57), leucine (6, 9, 16, 25, 33, 34, 35, 44, 57, 84, 88, 94, 96), phenylalanine (33, 34, 35, 57), tyrosine (16, 17, 25, 33, 57), aspartic acid (9, 23, 33, 34, 35, 84, 94), glutamic acid (33, 34, 35, 85a), and asparagine [4, 6, 7, 8, 13, 17, 18, 22, 23, 25, 27, 28, 32, 33, 34, 35, 44, 45, 50, 56, 57, 65, 66, 67, 68, 69, 70, 71, 77, 78 (not seen; cited by Oltmanns on p. 157 of "Morphologie und Biologie der Algen"), 82, 83, 85a, 88, 94, 96, 101] will all satisfy more or less satisfactorily the nitrogen requirement of one to many species. Occasionally, however, one or more of these compounds has proved to be non-assimilable or even toxic to some organisms. Two investigators (33, 35, 66) have reported organisms to which all of the amino acids mentioned above (except cystine, which was not tested) were unavailable, while others (11, 12, 16, 28, 32, 34, 43, 48, 54,

⁵ For the suggestion of this possibility the writer is indebted to Dr. Oran Raber, who has done work on this subject.

TABLE 1. Nitrogen absorbed from various nitrogenous compounds by *Chorella* sp. (F. B. Warr's No. 11).
(% in terms of $\text{KNO}_3 = 100$.)

Sources of nitrogen: other modifications of the culture solution	1st exper. mg. %	2nd exper. mg. %	3rd exper. mg. %	4th exper. mg. %	5th exper. mg. %	6th exper. mg. %	N-absorbed mean %
Potassium nitrate							
Usual conc.	27.5 ^a 103	10.2 100	10.7 100	3.7 ^a 100	4.9 100	5.6 100	100
1/2 conc.				3.4 ^b 92			92
With ppt. chalk			15.6 146	3.8 102			124
Potassium nitrite							
1/10 conc.			3.1 ^b 29				29
1/50 conc.			1.0 ^b 10				10
1/10 conc. + 9/10 KNO_3 ..			7.6 ^b 71				71
1/50 conc. + 49/50 KNO_3 ..			10.1 ^b 95				95
Ammonium sulphate							
Usual conc.	35.1 128			4.8 129	7.1 143		134
1/2 conc.				2.5 ^b 67			67
1/2 conc. + 1/2 KNO_3 ..					8.5 173		173
Ammonium chloride							
Usual conc.	35.8 131			2.9 ^b 77	8.1 164		124
1/2 conc.				2.9 ^b 78			78
1/2 conc. + 1/2 KNO_3 ..					8.2 166		166
Diammonium phosphate							
Usual conc.	14.5 ^b 53				10.3 208	7.7 137	133
With ground marble						10.4 184	184
Ammonium carbonate							
Usual conc.	49.3 ^b 179			3.1 84			132
1/2 conc.				5.9 157			157
Ammonium formate							
Usual conc.	26.9 98				4.3 87	3.2 58	81
1/2 conc.						3.8 67	67
With ground marble						12.4 221	221
With 0.0125N CaCl_2 ..						0.3 ^b 4	4
With 0.0025N CaCl_2 ..						3.1 ^b 55	55
With 0.0125N MgCl_2 ..						3.2 ^b 56	56
With 0.0025N MgCl_2 ..						3.0 ^b 53	53
With 0.0125N AlCl_3 ..						0.7 ^b 13	13
With 0.00125N AlCl_3 ..						1.5 ^b 27	27
Ammonium acetate							
Usual conc.	0.0 ^b 0			13.7 ^{b,c}	-0.1 -2	No growth	0
1/2 conc.				4.9 ^{b,c}			
1/2 conc.						8.0 143	143
1/10 conc.						4.0 71	71
1/2 conc. + 1/2 KNO_3 ..					6.1 124		124
1/2 conc. + 1/2 NH_4 -oxalate					10.6 216		216
1/2 conc. + 1/2 NH_4 -citrate.					8.2 167		167
With ground marble, etc. ^d ..							0
Ammonium propionate ^e							0
Ammonium lactate						13.8 244	244
Ammonium oxalate							
Usual conc.	51.6 ^b 188			10.2 274	10.8 220	9.1 162	211
1/2 conc.				10.6 ^b 283			283
1/2 conc. + 1/2 KNO_3 ..					8.9 180		180
With ground marble						12.0 214	214
Ammonium succinate							
Usual conc.	12.2 45				12.4 251	17.6 ^f 312	203
1/2 conc. + 1/2 KNO_3 ..					9.0 183		183
						15.5 ^f 277	277
Ammonium tartrate							
Usual conc.	47.1 ^b 171				5.5 ^b 112	8.9 158	147
With ground marble						16.6 295	295
Ammonium citrate							
Usual conc.					12.2 248	12.4 221	234
1/2 conc. + 1/2 KNO_3 ..					10.6 216		216
With ground marble						18.7 332	332
Acetamide			22.5 210			5.6 100	155
Urea	26.5 97						97

TABLE 1 (Continued)

Sources of nitrogen: other modifications of the culture solution	1st exper.	2nd exper.	3rd exper.	4th exper.	5th exper.	6th exper.	N-absorbed mean %
	mg. %	mg. %	mg. %	mg. %	mg. %	mg. %	
Guanidine carbonate							
Usual conc.						12.5 222	222
Double conc.			18.3 ^b 172				172
1/2 conc.			6.6 ^b 62				62
1/37 conc.			0.8 ^b 8				8
Double conc. + KNO ₃			21.4 ^b 201				201
1/2 conc. + KNO ₃			12.6 ^b 118				118
1/37 conc. + KNO ₃			11.6 ^b 108				108
Uric acid		Growth ^g				11.7 209	209
Hydroxylamine hydrochloride							
Usual conc.			No growth				0
With ground marble						No growth	0
Glucosamine hydrochloride							
Usual conc.			No growth				0
With ground marble						No growth	0
Glycine	35.2 128					16.2 289	209
Alanine		Growth ^h				17.5 311	311
Valine		1.4 14 ⁱ				3.4 61	38
Leucine		3.8 39 ⁱ				6.2 111	75
Phenylalanine		5.4 55					55
Tyrosine		Growth ^g				Growth ^g	Growth
L-cystine		Little or no growth				Growth ^g	Growth
Aspartic acid.							
Usual conc.		No growth					0
With ground marble						9.5 170	170
Asparagine	24.3 ⁱ 89					14.4 256	173
Glutamic acid							
Usual conc.		No growth					0
With ground marble						9.1 161	161
Peptone (Fairchild's)							
1.5 gm. per liter	84.2 ^b 307						307
Egg albumen							
1.5 gm. per liter	Slight growth						Growth
Diphenylamine							
Usual conc.	No growth						0
1/100 conc. + KNO ₃				No growth			0
p-aminoazobenzene		No growth					0
Azobenzene							
Usual conc.	No growth						0
1/100 conc. + KNO ₃				No growth			0
Diazoaminoazobenzene		No growth					0
No nitrogen added	1.7 ^b	0.5	-0.1	0.0	0.2	0.4	

^a Mean of four cultures. ^b One culture only. ^c Culture ran much longer than most of the others. The analytical result is out of line with the qualitative observations and is doubtless too high. Not included in final average. ^d Duplicate cultures with ground marble and single cultures with CaCl₂, MgCl₂, and AlCl₃ as used with the formate, and at pH values at the close of the experiment ranging from 5.1 to 5.6 gave no growth in any case. ^e Two experiments, each in duplicate, at the usual concentration, a single culture each at 1/5 and 1/10 concentration, duplicate cultures with half propionate and half KNO₃, and cultures with ground marble, CaCl₂, MgCl₂, and AlCl₃ as used with the formate and acetate at pH values ranging from 5.3 to 6.0 gave no growth in any case. ^f There is some doubt about these high results, owing to the possibility of a slight fungous infection of the stock solution of succinate and owing to the failure to agree with the first experiment. ^g Not analyzed because of a precipitate which may have contained nitrogen. ^h Lost in analysis. ⁱ Value probably too low, owing to accident in analysis.

62, 74, 75, 83, 85a, 99) have made similar reports for one or more of them. Not many investigators have worked with known mixtures of amino acids. Such evidence as has been presented (16, 43, 66, 75) seems to indicate that mixtures are little, if any, better than the best component of the mixture used alone.

Peptone.—Fairchild's peptone was used. It supported an abundant growth and allowed a heavy absorption of nitrogen. This result is in harmony with that secured in most of the numerous previous experiments (4, 5, 6, 7, 8, 9, 13, 14, 15, 16, 17, 22, 23, 24, 25, 30, 31, 32, 34, 35, 38, 39, 41, 47, 50, 52, 54, 56, 59, 66, 67, 68, 69, 70, 71, 77, 82, 83, 84, 85, 85a, 87, 88, 94). However, as with most other nitrogen sources, there are organisms and circumstances in which it is non-assimilable (17, 25, 32, 35) or also toxic (1, 25, 40, 45, 54, 86, 93).

Egg albumen.—Only a very small amount of growth took place on egg albumen, and since the cells could not be separated from the coagulum, no quantitative figure can be given for the nitrogen assimilated. There was some turbidity, possibly caused by bacteria; and possibly also the bacteria made available to the algal cells the small amount of nitrogen they absorbed. In any case, egg albumen seems to be but a poor source of nitrogen for this organism. This result is in agreement with that of Bialosuknia (16) with *Diplospira* Chodat Bial., which did not grow on egg white.

Miscellaneous aromatic compounds.—The compounds to be considered in this group are diphenylamine, p-aminoazobenzene, azobenzene, and diazoaminobenzene. Of these the first and third proved to be definitely very toxic. The other two did not support growth in the usual concentration of the nitrogen carrier and it is very likely that they are also very toxic.

General considerations.—When the results of this study and of the previous ones cited are considered, it becomes clear that most algae can use a considerable number of compounds as sources of nitrogen. It is also clear that most of the materials considered, even though very favorable nitrogen sources for some species, are poorly available, unavailable, or even toxic to others. Likewise, a material available to a given organism under one set of conditions may be unavailable under another set. These results would be still more evident if the published information on the availability to algae of still other nitrogenous compounds were also considered. It is true that most of the species studied were unicellular Chlorophyceae, since these are the easiest ones to isolate in pure culture by the usual methods, and that studies are needed with other types of algae, but it does not appear likely that such an extension of the range of our in-

formation would change, except in detail, the statements just made.

In this connection it may be remarked that the results given above are confirmatory of the idea that in nature green plants do not need to depend on nitrification to make organic nitrogen assimilable. On the contrary, many or most of them are able to utilize it as soon as ammonification has occurred, or even sooner.

SUMMARY

Various nitrogenous substances were studied in an otherwise complete mineral medium for the assimilability of the nitrogen by a species of *Chlorella* (Wann's No. 11) in artificial light. The criterion used was the amount of nitrogen accumulated in the cells. Subject to a small uncertainty due to possible slight decomposition of some materials in autoclaving, the results seem to justify the following conclusions:

The nitrogen in potassium nitrate is utilizable by the organism.

The nitrogen in many ammonium salts, both inorganic and organic, is also available, appearing in general to be more so than that in potassium nitrate. Some of the organic compounds are particularly favorable sources of the element, appearing much better than potassium nitrate in this regard.

The ammonium salts of the fatty acids, however, seem to form an exception. The formate is approximately as good a source of nitrogen as potassium nitrate, the acetate is much poorer, and the propionate cannot be used at all. No higher member of the series was investigated. The cause of the lack of availability is not clear. Neither calcium, magnesium, nor aluminum chloride increased the availability.

Acetamide, urea(?), guanidine carbonate, uric acid, several amino acids, potassium nitrite in dilute solution, and peptone are suitable as sources of nitrogen.

Hydroxylamine and glucosamine hydrochlorides, even at suitable acidities, do not furnish available nitrogen.

Diphenylamine, p-aminoazobenzene, azobenzene, and diazoaminobenzene are non-assimilable; the first and third are definitely toxic; and the other two are probably so.

While calcium appears to be unnecessary to the organism calcium carbonate is sometimes helpful in ammonium salt and some other cultures. The benefit is probably due chiefly to a more favorable reaction of the medium rather than to a specific effect of the calcium.

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THE EFFECTS OF SODIUM CYANIDE AND METHYLENE BLUE ON OXYGEN CONSUMPTION BY NITELLA CLAVATA¹

Edward Ross

PRELIMINARY EXPERIMENTS by the author (Ross, 1934) dealing with the action of cyanide and methylene blue on *Nitella* cells have been continued. Oxygen consumption was measured by the Warburg manometric technique. Current theories have been discussed in the light of the respiratory mechanism of *Nitella*. A mathematical analysis of the quantitative data obtained has been applied to the questions of residual respiration in the presence of cyanide and methylene blue-cyanide antagonism.

Work by Warburg (1919), Emerson (1927), and Genevois (1927) indicates that the substrate is instrumental in determining the type of effect produced by cyanide on *Chlorella*. Under autotrophic conditions, it accelerated oxygen consumption; in 1 per cent glucose solution, inhibition of respiration was reported. However, Genevois demonstrated typical

inhibition for *Scenedesmus basiliensis* both in Ringer's solution and in glucose, but oxygen consumption was completely inhibited by cyanide only when the alga was under autotrophic conditions.

The literature dealing with the effects of methylene blue on green plant cells is relatively meager, while there is little concerned with the dye-cyanide antagonism. Watanabe (1932) found the autotrophic respiration of *Chlorella* to be accelerated more than 100 per cent by 5.0×10^{-4} M methylene blue, but he states that this was not proportionately additive to the cyanide increase. In this study of *Nitella*, experiments with various concentration combinations of cyanide and methylene blue were performed.

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APPARATUS AND METHODS.—*The manometric technique.*—For this investigation of oxygen consumption by *Nitella* tips, the simple type of Warburg (1923) apparatus was employed (see also Dixon, 1934). Carbon dioxide was absorbed by 20 per cent KOH in the inset cup of the vessel, the absorption facilitated by the use of filter paper rolls. The rate of shaking in a constant temperature (23°C.) water bath was 74 eight-centimeter oscillations per minute. Increase of the rate of shaking to 96 had no significant effect on the rate of oxygen consumption.

Methods of handling Nitella.—About twenty-four hours prior to use, the *Nitella* cells were obtained from a large outside pool. One to two hundred tips were collected in a liter beaker of pond water. These individual clusters of cells varied from about two to six millimeters in diameter. During the interval between collection and experiment, the beaker containing the cells was open in a dark room. Immediately before use in an experiment, the cells were washed in fresh pond water and several dozen tips at a time spread on filter paper for one or two minutes' drying. One gram wet weight was then put into each manometer vessel, followed by 5 cc. of pond water.

After each experiment, the KOH of the inset cup was first carefully removed, and then the tips from each vessel were rapidly washed into separate beakers with tap water and washed continually for several days. Microscopic examination was made to determine possible injury entailed by the handling or chemical treatment. Evidence of vitality (protoplasmic streaming, regular chloroplast arrangement, and turgor) was noted after twelve hours' washing for all experiments, except cells from 10⁻² M or more dye or NaCN. Death resulted after three or four days for cells which had been in a combination of 10⁻³ M each of NaCN and methylene blue. A clear-cut picture of injury to wheat seeds was not evident even in 10⁻¹ M NaCN, as judged by subsequent germination following washing.

Standard procedure used.—After an experiment the bathing solution of the *Nitella* cells was pipetted off for pH determinations, using a glass electrode. Neutralization of the cyanide in 10⁻³ M solution was not found to influence the inhibition values significantly. With concentrations of NaCN greater than 10⁻³ M, it was necessary to neutralize in order that the pH after the experiment be less than 8.0.

In order to assure comparable results, a standard procedure was followed. The manometer vessel, as well as the capillary stem beyond the first bend, was always enclosed in an opaque black cloth in order to prevent photosynthesis by this green plant. Experiments were generally run in six-hour periods, split into three two-hour units as follows: (1) Determination of the normal O₂ consumption for the tips in each vessel. (2) Determination of the effects of a single reagent. The vessel was disconnected from the manometer for introduction of 0.4 cc. of a predetermined concentration of the reagent whose effect was to be established. This was done to obviate

diffusion of HCN from NaCN² solution in the side-arm into the vessel which affected the normal O₂ consumption. Methylene blue³ solution, freshly prepared, was also introduced by disconnecting the vessel. (3) Investigation of the effects of one substance added after the results for another had previously been determined—e.g., methylene blue added after cyanide.

In every case the value for oxygen consumption during the last hour of a two-hour unit was used in the data reported. The Q_{O₂} (mm.³ of oxygen consumed/hour/gm.) for both the half-hour preceding and following was never found to be very different from the last hour value as stated above; this applied to the experimental and control cases alike. It might be construed to represent a quasi-equilibrium value—i.e., after possible irritation by handling had worn off and before the effect of prolonged experimentation was evidenced.⁴

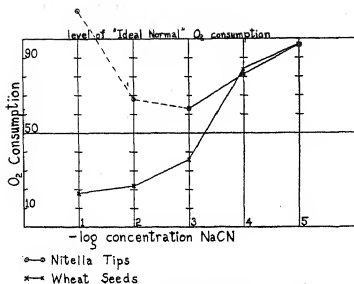
System of control corrections.—The experiments were set up as follows: One manometer and vessel served as the thermo-barometric control, containing 5.0 cc. pond water in the vessel and 0.4 cc. 20 per cent KOH in the inset cup. Another contained 1.0 gm. wet weight⁵ of *Nitella* tips, 5.0 cc. pond water, 0.4 cc. 20 per cent KOH in the inset cup, and served as a control for normal oxygen consumption. The remaining four were experimental, with exactly the same contents as the *Nitella* control for the first two hours of the usual six-hour run. The second two-hour unit was used to determine the effect of one of the chemical agents alone—e.g., 0.4 cc. of NaCN added to two of the experimentals (A and B). The third two-hour unit served to establish the effect of methylene blue added after cyanide, in this example: 0.4 cc. added to B and C, and 0.8 cc. of cyanide plus dye (mixed equal parts before adding) to the remaining experimental, D. Variations from this example included the using of two controls to study one particular effect, and changing of the time of addition of the dye from the last to the second two-hour unit, thus determining the effects of adding cyanide two hours after methylene blue.

Suppose NaCN were added to vessels A and B in a concentration such as to make 10⁻³ M when diluted by the pond water. To determine the effect of 10⁻³ M NaCN alone, then, one would compare oxygen consumption values for the second and the fourth hour of the run, as found for these two manometers. If it decreased 44 per cent in that time, while the con-

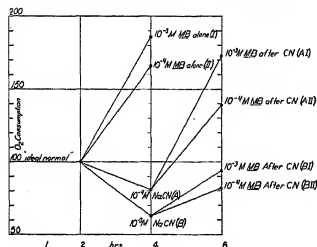
² Baker's Analyzed; assay (as NaCN) 96.8 per cent.

³ Merck's U.S.P.X., Medicinal; considered to contain 12 per cent H₂O of hydration.

⁴ As a rule the inhibition was noted to decrease about 2 per cent to 3 per cent an hour, especially after more than two hours exposure to cyanide. Two analyses made for cyanide in the bathing solution of *Nitella* showed that the concentration of that anion was reduced nearly fifty per cent from 10⁻³ M solution in three hours, no distinction being attempted between the amount absorbed by the KOH and that taken up by the *Nitella* cells.



O₂ Consumption by Nitella clavata
Effects of Methylene Blue Added Two Hours After NaCN



O₂ Consumption by Nitella clavata
2a. NaCN and Methylene Blue 10⁻³ M Added Before Adding 2b. NaCN Added After the Dye

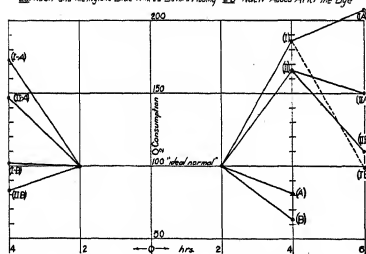


Fig. 1 (above). Average experimental values (scaled to 100) plotted against the negative log of the cyanide concentration for the oxygen consumption of *Nitella* tips and wheat seeds (Big Club). A total of thirty-two values is incorporated in the above figure; four O_2 consumption units is the average deviation from the mean values as plotted.

Fig. 2 (center). Graph depicting the level of the rate of oxygen consumption in the experimental solutions. The connecting lines between points have no meaning in terms of a change of rate with time. Total of forty-one values incorporated in this figure; average deviation from the means plotted is seven oxygen consumption units.

trol for the normal dropped 7 per cent, the inhibition due to cyanide would be 44 per cent — 7 per cent = 37 per cent. Scaled to the "ideal normal" of respiration taken at 100, the arbitrary value for this effect was considered to be $100 - 37 = 63$ (fig. 1). For methylene blue the drop of the control of course would be added to the acceleration as measured (C). If cells which had been in 10^{-3} M NaCN for two hours (B) showed, during the second hour in 10^{-3} M solution of methylene blue (6th hour of the run), an increase amounting to 55 per cent of the inhibited value, 63, while the oxygen consumption in the NaCN control A increased 5 per cent during the same time, then the result of the dye added after cyanide would be 63 plus $(55 - 5)$ per cent of 63 = 94 (fig. 1). Hence, the effect of methylene blue added after cyanide has been corrected, for the normal deviation (shown by A) from the inhibition established in the fourth hour of the run. The independent activity of the dye in the presence of cyanide is sought by this means of applying control corrections. The results from all the other combinations studied were treated in like manner.

EXPERIMENTAL RESULTS AND DISCUSSION.—General observations of respiration.—The normal Q_{O_2} (m.l./hr./gm.) of *Nitella* tips was usually found to lie between 20 and 30 in more than a hundred experiments.⁵ No seasonal variations were detected. The supply from the outside pond was generally insufficient for experiments only during the early fall. The Q_{CO_2} was determined in six experiments by comparing the pressure changes in two manometers run simultaneously, one containing KOH for CO_2 absorption and the other without KOH. The respiratory quotients were found to vary between 0.88 and 1.02.

Effects of NaCN.—Figure 1 shows the variation of oxygen consumption with concentration of cyanide for *Nitella* tips and wheat seeds. The seeds had been soaked in distilled water twenty-four hours prior to an experiment, otherwise the procedure and control corrections were the same as described for *Nitella*.

According to Warburg's (1928) original concept of the rôle of iron in cellular respiration, cyanide forms a chemical complex, resulting in reversible inactivation of the respiratory ferment. However, a cyanide-stable portion of normal aerobic respiration was soon found to be of common occurrence (Dixon and Elliott, 1929). Robinson (1924) showed that the strong catalytic action of some hematin compounds on the oxidation of linseed oil was not checked by potassium

⁵ One gm. wet wt. reduced to about 0.025 gm. after drying to constant weight at $100^\circ C$. Seven duplicate samples (wet wt.) used in early experiments checked within 2 per cent by dry wts.; it was considered of no advantage to procure dry wts. thereafter.

Fig. 3 (below). Graph constructed on the same basis as figure 2. In this case, generally only one value determines the position of a point. Exceptions are points I-B, II-B and I A. Three values established the exceptional maximum of 206 for point I A. I and II indicate 10^{-3} M and 10^{-4} M methylene blue, respectively, while A and B represent 10^{-3} M and 10^{-4} M NaCN (cf. fig. 2).

cyanide of $1\text{M}/600$ concentration, which is about five times the concentration of iron in the catalyst. Kuhn and Meyer (1930) suggest that the cyanide-stable portion of respiration may be due essentially to fat metabolism. Either this oxidation of fats is normally simultaneous with cyanide-labile respiration, or it develops after cyanide has inhibited the carbohydrate metabolism below a minimum value. The work on autotrophic and starved plant cells shows that their oxygen consumption is relatively insensitive to cyanide. Heterotrophic conditions render the respiration cyanide-sensitive. Thus, it may be that when carbohydrate metabolism is reduced beyond certain limits, fat metabolism comes to play a prominent rôle in respiration.

The residual oxygen consumption in the presence of cyanide may depend upon the action which occurs between the respiratory enzyme and cyanide which does not allow complete inactivation because of equilibrium requirements. An analysis of the data was made from this viewpoint, based on Warburg's proposal of complex formation between the respiratory ferment and cyanide. The general reaction may be written: $a(\text{CN}) + b(\text{RF}) \rightleftharpoons (\text{CN})_a(\text{RF})_b$. Using the values found for the inhibition in 10^{-5}M and 10^{-4}M cyanide for both wheat seeds and *Nitella* tips, the solution (see CALCULATIONS) of the reaction becomes $(\text{CN}) + 3(\text{RF}) \rightleftharpoons (\text{CN})(\text{RF})_3$. If one now calculates the relation between the inhibition and the negative log of cyanide concentration, a curve very similar to that found experimentally for wheat seeds is obtained. The greatest deviation (about 15 per cent) occurs in the center of the curve. Equilibrium relations predict that 99 per cent inhibition requires 300 M cyanide. Complete inhibition of oxygen consumption could not be expected in vivo because of the equilibrium requirements. Therefore, it is not necessary to postulate an intrinsically cyanide-stable respiration to account for 15 to 20 per cent residual oxygen consumption.

Figure 1 seems to show some change in the respiratory mechanism of *Nitella* in 10^{-3}M cyanide. Analysis of the data indicates that an intrinsically cyanide-stable respiration comes into play in that concentration. However, it cannot be definitely concluded from these data that fat metabolism has appeared under conditions of inhibited carbohydrate metabolism. Using 37 per cent inhibition found for *Nitella* in 10^{-3}M NaCN as a basis for calculation, R (cyanide-labile part of total oxygen consumption) comes out as 85 instead of 100, leaving 15 per cent apparently cyanide-stable oxygen consumption. However, wheat seeds show inhibition in 10^{-3}M NaCN greater than that predicted theoretically, so that R comes to about 120. Yet R (calculated) decreases as the concentration increases, until in 10^{-1}M solution it is 95. Two generalizations may be made. (1) Irrespective of the actual values used here, the concept of an equilibrium between cyanide and the system inactivated by it can account for a significant residual respiration in the presence of the cyanide. It may be thus that an

apparent maximum inhibition is usually found with concentrations between 10^{-3}M and 10^{-2}M . (2) The appearance of a cyanide-stable respiration in the presence of the cyanide can account for the low maximum inhibition found for *Nitella* tips; this generalization not only is substantiated for wheat seeds, but also it has been used to explain the effects of methylene blue on *Nitella* in the presence of cyanide (post vide).

The effects of methylene blue.—Figures 2 and 3 show the effects of the dye by itself and also those of various combinations with NaCN. For a rigid examination of the system of control corrections as a means of obtaining absolute results, it is of importance to discover the effects of different orders of combination of the reagents. Tips exposed to 10^{-3}M methylene blue for more than two hours show, by the exceptional value of 206 (fig. 3b), the initiation of an in vivo process in the cell's respiration.

Blum (1935) describes a parallel result wherein photodynamic hemolysis by eosine and hemolysis by eosine with H_2O_2 is accelerated by $5 \times 10^{-4}\text{M}$ NaCN, while $5 \times 10^{-2}\text{M}$ cyanide inhibits these processes. Several studies (Wohlgemuth and Szörenyi, 1933) describe an acceleration of oxygen consumption in photosensitized tissues by 10^{-4}M and 10^{-3}M HCN. If the oxidation of cell components is an operative factor in photodynamic action, the inactivation of catalase in dilute cyanide would allow H_2O_2 to accumulate in the cells, increasing the rate of oxygen consumption and accelerating hemolysis.

The production of H_2O_2 in the autoxidation of methylene blue (Warburg, Kubowitz, and Christian, 1930) may occur in two hours' exposure of *Nitella* cells to 10^{-3}M dye. This seems to be similar to eosine plus H_2O_2 in hemolysis—the so-called "dark reaction" of photodynamic action. When 10^{-4}M cyanide is added after the dye, an increase of oxygen consumption may occur, due to inactivation of catalase, because: (1) the reaction, $\text{leuco-dye} + \frac{1}{2}\text{O}_2 = \text{dye} + \text{H}_2\text{O}$, is replaced by: $\text{leuco-dye} + \text{O}_2 = \text{dye} + \text{H}_2\text{O}_2$; (2) accumulation of H_2O_2 brings about, or increases, a special oxidative process, perhaps of a destructive nature. The data support the second possibility, though not exclusively. An injury of a delayed nature—i.e., cells washed four days after 10^{-3}M each of dye and cyanide—tends to favor the second hypothesis.

The action of methylene blue on cellular respiration has been explained both from the viewpoint of a direct dehydrogenation process (Szent-Györgyi, 1924) and on the basis of the dye's action through an intermediate substance (Warburg, Kubowitz, and Christian, 1930). If the dye acts in the manner first mentioned, it may be considered to be reduced directly by the cell's dehydrogenase-substrate systems. On the other hand, Warburg has proposed that methylene blue is reduced by an Fe enzyme, which in turn is reduced by the dehydrogenase-substrate systems. Experimental results are compared in table 1 with values calculated on the basis of these hypotheses

separately. That which affords the better agreement is sought in the analysis of the comparison which follows.

In 10^{-4} M NaCN, the oxygen consumption of *Nitella* tips was inhibited 19 per cent, while 10^{-4} M methylene blue alone increased the oxygen consumption 66 per cent. If the dye oxidized the substrate directly, it may be considered to react with active hydrogen in the ratio of 66 to every 100 normally oxidized by the cell's oxygen-activation system. Since an increase in the concentration of the dye results in a greater acceleration than 66 per cent, more active hydrogen remains than the cell's system plus the dye can oxidize. The methylene blue must be submaximal in concentration, increasing oxygen consumption even in the presence of cyanide just as when alone. In 10^{-4} M each of NaCN and methylene blue, this hypothesis predicts a value of $166 - 19 = 147$ (cf. table 1).

the value for the oxygen consumption in 10^{-3} M NaCN and 10^{-4} M dye would be as follows: $85 =$ cyanide-labile oxygen consumption; $37 =$ inhibition in 10^{-3} M cyanide; $85 - 37 = 48 =$ part acted on by the dye; $48 \times 0.66 = 32 =$ increase due to the dye (10^{-4} M); $100 - 37 = 63 =$ oxygen consumption in 10^{-3} M NaCN alone; $63 + 32 = 95 =$ oxygen consumption in combination. Values calculated in this manner are shown in the last column (f) of table 1. They are in better agreement with the experimentals for 10^{-3} M cyanide than the other calculated values.

To conclude definitely on the mode of action of methylene blue from this analysis would be premature. Certainly direct dehydrogenation of the substrate cannot explain the data sufficiently, nor are reservations apparent through which such an explanation can be made. However, on the basis of the dye's action through an intermediate Fe system, a suitable

TABLE 1. Comparison of average experimental values taken from figures 2 and 3, with values calculated as described in the text. Values represent oxygen consumption scaled to "Ideal Normal" of 100 in various combinations of NaCN and methylene blue.

Concentration		Experimental			Calculated		
NaCN	Dye	A	B	C	Dye-substrate d	Dye-iron-substrate e	f
10^{-4} M	10^{-4} M	139	146	151	147	135	—
10^{-4} M	10^{-3} M	173	173	(206)	167	151	—
10^{-3} M	10^{-4} M	82	83	110	129	105	95
10^{-3} M	10^{-3} M	94	102	99	149	117	104

Explanation of columns: A: Average experimental values found when the dye was added two hours after NaCN.—B: When the NaCN and dye were mixed prior to addition to *Nitella* environment.—C: When the NaCN was added two hours after the dye.—Dye-substrate: Values calculated on the basis of the action of methylene blue by direct dehydrogenation of the substrate.—Dye-iron-substrate: (e) Calculated values on the basis of the dye's activity through an iron-containing system of the respiratory chain. (f) Calculation on the basis of 15 per cent cyanide-stable oxygen consumption in the presence of 10^{-3} M NaCN.

The concept which pictures the dye's action through an intermediate substance involves the valence shift of iron. In this case, inactivation by cyanide must be considered to inhibit not only oxygen consumption via the cell's normal system, but also the increase that would be caused by methylene blue alone. Thus, since 10^{-4} M dye accelerates 66 per cent and cyanide alone inhibits 19 per cent (10^{-4} M), one would expect a value of $(100 - 19) \times 1.66 = 135$ for the oxygen consumption in a mixture of the two. According to this hypothesis, if cyanide were in sufficient strength to give complete inhibition alone, the presence of methylene blue would not afford an alternative path for oxygen consumption in vivo.

Referring to table 1, it is especially noteworthy that the experimental results show close agreement with the calculated values of the first column (d) for the more dilute cyanide, while better agreement is obtained with values in column (e) for 10^{-3} M NaCN. As was noted before, in that concentration of cyanide the analysis of the data suggested that a 15 per cent cyanide-stable respiration came in play. If that were the case, one might expect the activity of methylene blue to be further limited. Thus, the calculation of

agreement of experimental values with calculated ones can be obtained for 10^{-3} M NaCN. Furthermore, it seems reasonable to suppose that the catalytic activity of the dye through iron might be inhibited only when the amount of active iron is a limiting factor, a factor not reached by inactivation in 10^{-4} M cyanide, but becoming effective in 10^{-3} M solution. With this qualification, then, the data are amenable to explanation on the grounds of Warburg's hypothesis for the mode of action of methylene blue.

SUMMARY

The respiration of *Nitella clavata* was studied by the manometric method. Actively growing tips of this coenocytic green alga were used. Supplementing experiments with wheat seeds (Big Club) were also performed.

An inhibition of 20 per cent in 10^{-4} M NaCN and an acceleration of 66 per cent in 10^{-4} M methylene blue were observed for *Nitella*. The maximum inhibition of the oxygen consumption of the alga was found to be about 40 per cent, while that for wheat seeds was more than 80 per cent.

The relationship between the negative log of cyanide concentration and oxygen consumption found experimentally for wheat seeds can be closely predicted by calculation. It was noted that chemical equilibrium conditions alone allow a 15 to 20 per cent residual oxygen consumption in the presence of 0.1 M NaCN. However, calculations were made which suggest that a 15 per cent cyanide-stable oxygen consumption may come into play for *Nitella* tips in 10^{-3} M cyanide.

The question of the antagonistic action between cyanide and methylene blue was considered on the basis of the dye's activity in two ways. If the methylene blue is a direct hydrogen acceptor for the dehydrogenase-substrate systems, its effects would not be quantitatively influenced in the presence of cyanide. On the other hand, the dye may be reduced by the cell's normal iron enzyme systems. In the presence of cyanide, then, methylene blue activity would be reduced. Quantitative analysis of the data for *Nitella* tends to favor its action through an intermediate system, rather than directly on the dehydrogenase-substrate systems.

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CALCULATIONS

Let all values for oxygen consumption be scaled to 100 as previously described.

Let I = that part of the total oxygen consumption which is inhibited by any one concentration of cyanide (CN).

R = the maximum part of the total oxygen consumption which can be checked by CN.

The system inactivated by cyanide is indicated by RF, and the small letters such as a, b, k , etc., represent constants.

Then, in the equilibrium:

$$\begin{aligned} a(\text{CN}) + b(\text{RF}) &\rightleftharpoons ((\text{CN})_a (\text{RF})_b) \\ I &\propto ((\text{CN})_a (\text{RF})_b) = c((\text{CN})_a (\text{RF})_b) \\ (R - I) &\propto (\text{RF}) = d(\text{RF}) \end{aligned}$$

and:

$$k' = \frac{((\text{CN})_a (\text{RF})_b)}{(\text{CN})^a (\text{RF})^b} = \frac{(1/c) I}{(\text{CN})^a (1/d)^b (R - I)^b}$$

incorporating constants and converting to logarithms:

$\log k' = -a \log(\text{CN}) + \log I - b \log(R - I)$
taking experimental values for the variables, I at two concentrations of CN, viz., 3 and 18 in 10^{-5} M and 10^{-4} M respectively; assuming the total oxygen consumption cyanide-labile, $R = 100$: $-a \log 10^{-5} + \log 3 - b \log 97 = -a \log 10^{-4} + \log 18 - b \log 82$

$$a = \log 18/3 + b \log 97/82 = 0.78 + 0.07 b$$

then, if $b = 3$, $a = 0.99 = 1$
solving for $\log k'$:

^a Assuming a 50 per cent absorption of HCN (cf. footnote 4), $\log k' = -0.18$. This shifts the entire theoretical curve of $I/\log(\text{CN})$ by 0.30 of a log unit, the conclusions as made in the text remaining essentially the same. If a similar correction ($I = 37$, in 0.5×10^{-3} M NaCN) is made in the calculation of R , its value becomes 73 instead of 85, indicating an even greater cyanide-stable portion.

$$\begin{aligned} \log k' &= -\log 10^{-4} + \log 18 - 3 \log 82 \\ &= 4 + 1.25 - 5.73 = -0.48 \end{aligned}$$

Calculating values for (CN) at different I values:

$$\begin{aligned} \log(\text{CN}) &= 0.48 + \log I - 3 \log(R - I) \\ \text{when: } I &= 50, \log(\text{CN}) = -2.9 \\ &= 70, \quad \quad \quad = -2.1 \\ &= 80, \quad \quad \quad = -1.5 \\ &= 99, \quad \quad \quad = 2.5 \end{aligned}$$

Calculating values for R :

$$\begin{aligned} \text{when } (Nitella) I &= 37, (\text{CN}) = 10^{-3} \text{ M} \\ \log(\text{CN}) &= 0.48 + \log 37 - 3 \log(R - 37) \\ \log(R - 37) &= 1.68 \\ R &= 48 + 37 = 85 \\ \text{when (wheat seeds) } I &= 64, (\text{CN}) = 10^{-3} \text{ M:} \\ R &= 121 \\ \text{when (wheat seeds) } I &= 82, (\text{CN}) = 10^{-1} \text{ M:} \\ R &= 95 \end{aligned}$$

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INFLORESCENCE PATTERNS AND SEXUAL EXPRESSION IN *BEGONIA SEMPERFLORENS*¹

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STUDIES of sexual expression in the plant kingdom have been concerned especially with genera and species that are partly or completely dioecious. This is true of the lower groups and applies to the flowering plants as well, even though with them rigid separation of the sexes in the mature plants is distinctly exceptional. In *Begonia semperflorens*, Link & Otto, staminate and pistillate flowers normally occur not merely on the same plant, but in the same inflorescence. Since the flowers bear but one type of sporophyll with remarkable constancy, this species in its monoecism may well illustrate one of the stages preceding dioecism.

In angiosperms dioecious to varying degrees it has been demonstrated repeatedly that the nature of the flower may be influenced by external conditions. Accordingly, *Begonia semperflorens* was studied as a form suitable to cast light upon two problems. The first is the determination of the number and distribution of staminate and pistillate flowers within individual inflorescences and the analysis of the external architecture of the resulting patterns in such flower clusters. The effect of soil fertility, as one of the environmental factors, on the production of the two kinds of flowers, here in the same plant and inflorescence, in comparison with dioecious forms, constitutes the second.

LITERATURE REVIEW.—The general characters of the inflorescence and flowers of *Begonia* were considered briefly in the older literature by Wydler (1851, 1878), and the family was treated in monographic form by Klotzsch (1854). Eichler's (1878) classical presentation gave a brief résumé of some of the cardinal features of the flowers and inflorescence, including a partial diagram of the inflorescence of *Begonia semperflorens*. The general validity of the cincinnus as a fundamental type in this genus was strongly questioned by Benecke (1882), but Eichler's conclusion has since been supported in the extensive consideration of both the racemose and cymose types of inflorescences of this family by Irmischer (1914). Goebel (1910) considered the floral variations at some length, suggesting, with Benecke, that the four-parted perianth of the staminate flowers is derived from the five-parted type illustrated by the pistillate. He also knew the cymose kind of inflorescence. The flowers were further considered by Sandt (1921). Digests of the extensive literature have been given by Warburg (1894), and more recently in comprehensive fashion by Irmischer (1925). Unusual flowers of *Begonia*, bearing both stamens and pistils, have been described by Hooker (1860), Crocker (1861), Masters (1869), Müller (1870, 1871), Magnus (1884),

Darwin (1890), Dümmer (1912), Bond (1915), Worsdell (1916), and Irmischer (1924).

Irmischer (1924) has made a special study of the distribution of staminate and pistillate flowers in the monoecious *Begonia Wallichiana*. Of the four flowers of an inflorescence in this species the first three are normally staminate, the fourth is pistillate. A plant brought indoors from the greenhouse at the Botanical Museum at Berlin formed two staminate and two pistillate flowers to an inflorescence, the two staminate being produced first. Intermediate flowers with stamens and pistils variously developed also occurred. These changes, he thinks, were associated with environmental conditions.

Bateson and Sutton (1919) found that *Begonia Davisii*, a single-flowered wild type, gave all double-flowered offspring in the staminate flowers when it was used as a pollen parent in crosses with other double-flowered forms. This indicates, according to the authors, that in this single-flowered wild type the pollen carries doubleness. The male side of this species, they believe, bears doubleness exclusively.

According to Pastrana (1932), the normal diploid chromosome number of *B. Schmidtiana* is thirteen, but the staminate flower has only twelve; the haploid number of the microspores is six, while that of the functional megaspore and egg is seven. In *B. incana*, on the other hand, Mereminski (1936) found that the haploid chromosome number in both kinds of flowers is fourteen, with no chromosomal difference between the sexes. Heitz (1927) reported fairly large and varying chromosome numbers in numerous species of *Begonia*.

The more important general literature dealing with form and its determination in the plant kingdom has been previously reviewed (Matzke, 1929, 1932). In a series of investigations on the algae and lower fungi Harper (1916, 1926, 1929) has emphasized the importance of adjustments between such factors as mutual pressure, adhesion, surface tension, inherited form tendencies of the cells, and functional hypertrophy in form and development. Sinnott (1935, 1937) has demonstrated the importance of shape genes in controlling relative growth rates and has stressed the significance of correlated growth rates in form-determination. Angles of branching in trees have been very briefly discussed by Murray (1927), and Penrose (1925) sought to establish, in the growth rate of the whole plant as well as in certain parts, phases of increase, constancy, and decrease. Studying the gynomonoeious *Satureia hortensis*, Correns (1907, 1908) was able to establish a fairly definite pattern in the formation of pistillate and perfect

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flowers on a single plant. This was affected, to some extent, by environmental conditions.

The question of sex has been treated at great length in biological literature. The various concepts and explanations of sexual expression seem to hold in particular cases. There are the factorial interpretations given for the higher plants especially, among others, by Correns (1928); the similar but simpler and exceedingly convincing factorial analysis that Allen (1919, 1932, 1935) has applied to dioecious bryophytes. These may or may not be correlated with visible chromosomal differences between the sexes.

The influence of environmental conditions has been emphasized by Schaffner (1921, 1922, 1923, 1927, 1935) and numerous other workers, on such plants as *Cannabis sativa*, *Arisaema triphyllum*, *Arisaema Dracontium*, *Humulus japonicus*, *Zea mays*, *Thalictrum dasycarpum*, and *Urticastrum divaricatum*. By making incisions and thus controlling the nutriment of various parts of the plant, Lewitsky (1925) found that well nourished branches of *Veratrum* produce perfect flowers, while poorly nourished ones, on the other hand, have staminate flowers with abortive pistils. Stout (1923) states that "sex of flowers is determined progressively as they are formed in response to regulation by internal biogenetic conditions."

More strictly chemical interpretations associated with sex have been advanced by various investigators. Gardner (1923) found that high carbohydrate content favors the production of perfect flowers in the strawberry, while low carbohydrate content induces the development of pistillate flowers. Talley (1934) similarly found that staminate plants of hemp have higher average percentages of carbohydrates than pistillate plants, but that nitrogen is relatively more abundant in the pistillate plants. Studying hydrogen-ion concentration in *Lychnis dioica*, Stanfield (1937a, 1937b) reported that in general the stems of staminate plants are slightly more alkaline than those of pistillate plants, though the expressed sap of the staminate plants in the actively blooming stage is more acid than that of the pistillate plants. Differences in acidity between the two types of plants were not marked. According to Camp (1929, 1932), certain staminate flowers or plants are associated with greater catalase activity than the pistillate. Oxidase activity of the two sexes varies in *Lychnis dioica*, according to the findings of Stanfield (1937b). Summaries and discussions of the literature of sex are given by Correns (1928), Joyet-Lavergne (1931), Robbins and Pearson (1933), Bressman (1934), Stanfield (1937b), and by many others. While it is possible to consider the results of investigators of sex in the plant kingdom from the standpoints of factorial analyses, of environmental influences, and of chemical correlations (including hormones), these distinctions are arbitrary; the overlapping of the last two is immediately obvious, and of either of these with the first only somewhat less so.

FLOWERS.—All the plants used in the present study were grown from seeds purchased commercially. Four varieties were utilized: *Begonia semperflorens* dwarf vernal, *B. semperflorens erfordia*, *B. semperflorens prima donna*, and *B. semperflorens luminosa*. The two former were purchased as varieties of *B. semperflorens*, the two latter as varieties of *B. gracilis*. Fotsch (1933) points out that *B. semperflorens* has been crossed with *B. gracilis*. Presumably these two varieties—namely, *B. semperflorens prima donna* and *B. semperflorens luminosa*—are hybrids. They are included here as varieties of *B. semperflorens* and may be considered as representatives of the *gracilis* class of that species (Fotsch, 1933). They differ markedly in several important respects from *B. gracilis* (DeCandolle, 1864), especially in having a much-branched stem, in lacking completely the bulblets in the axils of the leaves, in not having tuberous roots, and in having the stamen filaments free. In the present work, therefore, they are not included in the species *B. gracilis*, but as varieties of *B. semperflorens*.

In *B. semperflorens* the staminate and pistillate flowers are quite distinct, though both are normally found in the same inflorescence, as shown in figure 32, in which the four upper flowers are staminate and the four lower are pistillate. Each staminate flower ordinarily consists of four perianth parts decussately arranged, the outer pair being distinctly larger, and of numerous stamens, about 32–46. Each node of the inflorescence at which the staminate flowers are developed also bears two bracts. The pistillate flower has a five-parted quincuncially arranged perianth and an inferior tricarpeillary ovary which is three-winged, one wing being decidedly larger than the others. The three stigmas are each bifid and spirally twisted. Three bracts usually occur at the base of the ovary of each pistillate flower, alternating with the three wings. Benecke (1882) believes that these originate as two, one of which subsequently becomes split.

Although references to flowers with both stamens and pistils are not infrequent in the literature, only a single flower showing unmistakably both stamens and carpels was found. Its position was that of a staminate flower in the inflorescence; it had four perianth parts, two of which were smaller and inside of the two larger outer ones; one of the latter was two-lobed and somewhat larger than the other, but not nearly double in size. There were sixteen stamens, of which all but one were well developed and contained pollen. The gynoecium consisted of a superior tricarpeillary pistil with three two-lobed stigmas showing the normal spiral twist, and a somewhat abortive ovary, of which one carpel was open and showed the exposed ovules. This flower was staminate in its position, in its perianth (though the two-lobed petal suggested that of a pistillate flower), and in possessing approximately half of the normal complement of stamens. In place of the other stamens a superior tricarpeillary pistil was developed. Although the flowers listed below were not scrutinized with care

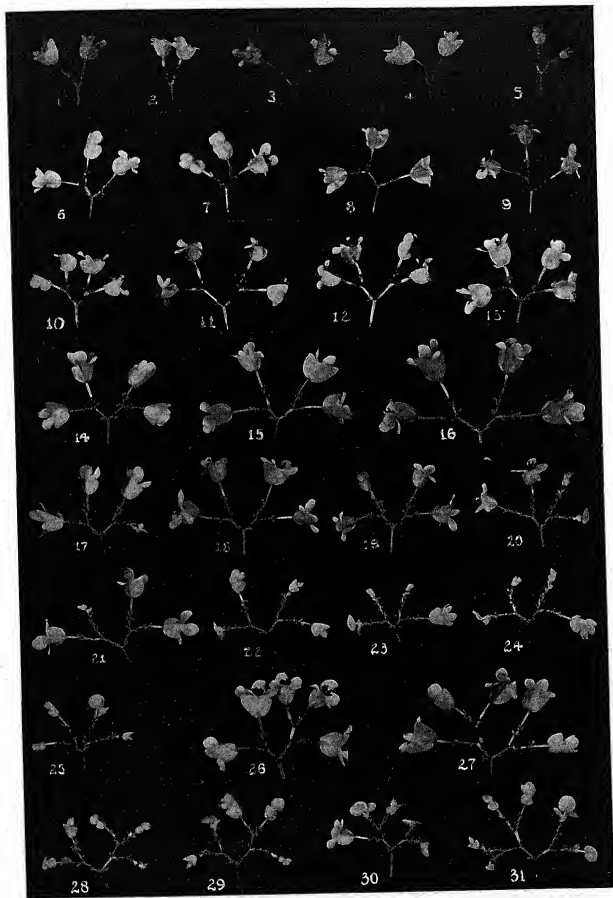


Fig. 1-31. Inflorescences of *Begonia semperflorens erfordia* with varying numbers of pistillate flowers: 2 in fig. 1-5, 3 in fig. 6-9, 4 in fig. 10-25, 5 in fig. 26-30, 6 in fig. 31. Most of the staminate flowers have dropped off, though some are still in position in fig. 25, 29, 30, and 31. The positions of the staminate flowers that have fallen are represented by the paired bracts visible in some cases.

TABLE 1. Analysis of 258 dichotomies in inflorescences of *Begonia semperflorens* *erfordia*.

	1 Primary as well as final di- chotomies	2 Primary but not final di- chotomies	3 Total primary dichot- omies	4 Secondary as well as final di- chotomies	5 Secondary but not final di- chotomies	6 Total secondary dichot- omies	7 Tertiary, all final dichot- omies	8 Totals
FIRST INTERNODE OF								
1 Paired branches equal	6	17	23	34	0	34	1	58
2 Paired branches unequal ...	8	75	83	104	7	111	6	200
3 Paired branches equal, branches with equal num- ber of flowers	2	5	7	25	0	25	0	32
4 Paired branches equal, branches with unequal, number of flowers	4	12	16	9	0	9	1	26
5 Paired branches unequal, longer branches with more flowers	3	30	33	53	6	59	5	97
6 Paired branches unequal, shorter branches with more flowers	0	34	34	10	0	10	1	45
7 Paired branches unequal, branches with equal num- ber of flowers	5	11	16	41	1	42	0	58

for the presence of carpels or stamens in flowers of the opposite type,—and some of the staminate flowers especially drop off before they open,—the finding of only a single flower with both kinds of organs well developed indicates that in the plants studied this is a rare occurrence. Several cases of more extensive modification of staminate into pistillate flowers occurred in which only pistils were present. Other abnormalities, such as supernumerary or missing petals, a bicarpellary instead of a tricarpellary ovary, a tetracarpellary ovary, and various degrees of reduction or abortion of the complete flower were found.

INFLORESCENCES.—In *Begonia semperflorens* the inflorescences usually begin as axillary dichasia, which develop into cincinni (uniparous scorpioid cymes) after one or several forkings. The cincinnus may be looked upon as a modification of the dichasium, in which one instead of the two branches at each node develops, and the branches at successive nodes are on alternate sides. Figures 1 to 31 are photographs of inflorescences of *B. semperflorens* *erfordia*, illustrating the dichasial and cincinnal development. For the most part only the pistillate flowers in these photographs remain, the staminate having dropped off. Figures 1A to 31A are diagrams of these same inflorescences in their entirety, in which each staminate flower is represented by a "♂" sign and each pistillate flower by a "♀" sign; figure 1A is the diagram of figure 1; figure 2A corresponds with figure 2, etc.

The first internodes of the two branches at the same node of the dichasium may be of equal length, as for instance at the first node of inflorescence 3 and 3A, 5 and 5A, 13 and 13A, 23 and 23A, 31 and 31A; or they may be of unequal length, as at the first node of inflorescence 2 and 2A, 4 and 4A, 6 and 6A, 7 and 7A, 8 and 8A, etc.

The length of the first internodes of the paired branches originating at the same node in the inflorescence was measured with dividers in 258 cases. In 58 of these the two internodes were equal in length or very nearly so, in 200 instances there was a noticeable difference, which was not infrequently very slight. The results of these measurements are given in table 1. Primary dichotomies are those occurring at the first node of the inflorescence and are represented in figures 1 to 31 and 1A to 31A. Primary as well as final dichotomies are represented in figures 1A, 2A, 3A, 4A, and 5A. Figures 6A to 31A have primary dichotomies at the first node, but they are not final as well, since one or both of the branches of the first node fork again. The dichotomies at the second node are each secondary. In figures 6A, 7A, 8A, and 9A there is one secondary dichotomy, while figures 10A to 31A each contain two secondary dichotomies. Figures 26A, 27A, 28A, 29A, and 30A each show one tertiary dichotomy, figure 31A shows two tertiary dichotomies, all of which are final as well, since no forkings occur beyond them. The term "dichotomy" is used here for simplicity; more accurately they may be spoken of as false dichotomies.

An examination of table 1, column 2, shows that if the primary dichotomies are not final, the relative length of the first internode is not correlated with the number of flowers, since the longer branch had more flowers in 30 cases, the shorter one had more in 34 cases. However, in the case of final dichotomies throughout the entire inflorescences, the longer internode had more flowers in 61 instances, the shorter had a greater number in only 11 instances, as is evident by the totals of lines 5 and 6 of columns 1, 4, and 7 in table 1. The final dichotomy therefore definitely foreshadows the cincinnus. For instance, in

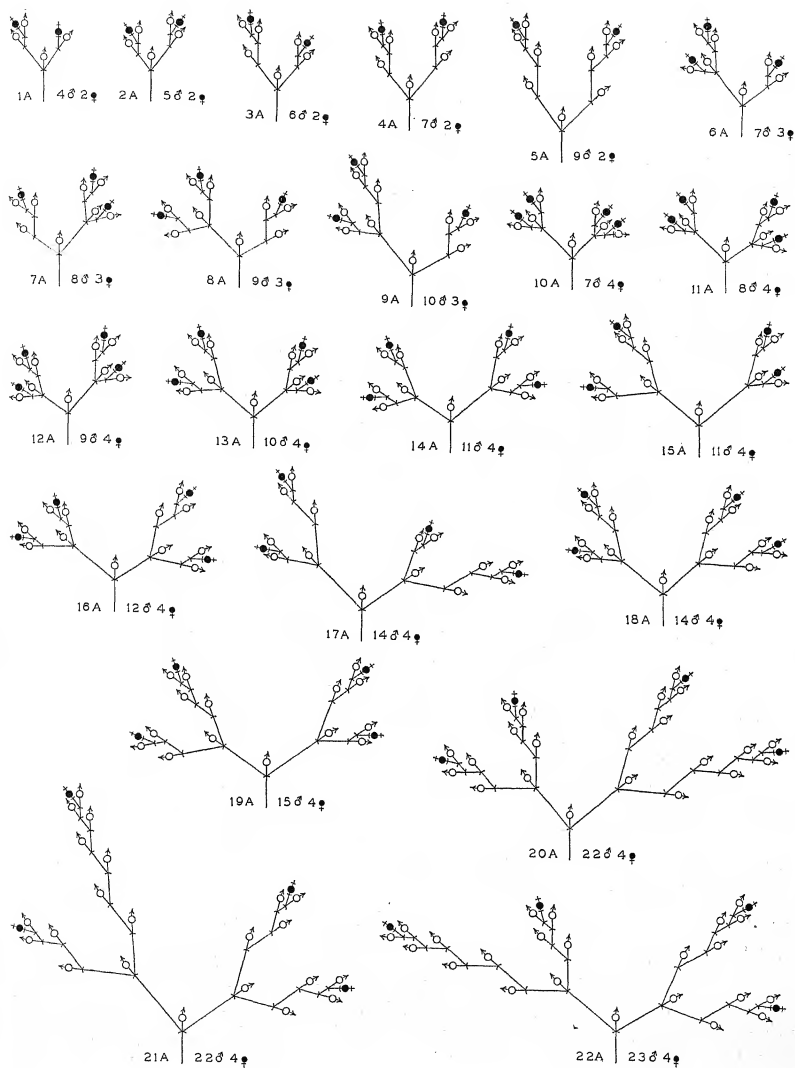


Fig. 1A-22A. Diagrams of inflorescences shown in fig. 1-22, each staminate flower shown in position by a "♂", each pistillate flower by a "♀". The numbers at the right of each figure indicate the number of staminate and pistillate flowers in each inflorescence.

figure 6A the secondary dichotomy on the left branch of the diagram has a larger upper branch which bears three flowers and a smaller lower branch which bears two. Similarly in figure 19A the secondary dichotomy on the right side has four flowers on its longer (upper) branch (as indicated by the length of the internode) and three on its shorter (lower) branch. The left side of that same diagram, 19A, is one of the exceptional cases in which the shorter (upper) internode supports five flowers, while the longer (lower) one has only four. It may be said then that the last dichotomy, before the dichasium changes into the cincinnus, definitely foreshadows the cincinnus; in the large majority of cases the two internodes

this point from the living material presents some obstacles, and the measurement of the angles in the photographs of inflorescences is open to criticism because of the three-dimensional branching of the inflorescences. To obviate these difficulties as far as possible, only measurements of primary dichotomies in the photographs of inflorescences were made, in which the two first internodes of each dichotomy showed a distinct inequality in length and in which the longer one bore (with successive branchings) at least two flowers more than the shorter (weaker) one. Twenty-two such cases were found, of which fourteen had the stronger branch forming a more acute angle than the weaker one with the extension of the main axis

TABLE 2. Frequency of occurrence of inflorescences represented in figures 1A-31A.

1	2	3	4	5
	Ratio of staminate to pistillate flowers	Number of inflorescences found	Diagram	Mirror image
Fig. 1A	4♂ 2♀	3		1
2A	5♂ 2♀	3	symmetrical	
3A	6♂ 2♀	4		2
4A	7♂ 2♀	3	symmetrical	
5A	9♂ 2♀	1	symmetrical	
6A	7♂ 3♀	8	4	4
7A	8♂ 3♀	8	3	5
8A	9♂ 3♀	9	5	4
9A	10♂ 3♀	2	1	1
10A	7♂ 4♀	1	symmetrical	
11A	8♂ 4♀	4	1	3
12A	9♂ 4♀	5	symmetrical	
13A	10♂ 4♀	11	6	5
14A	11♂ 4♀	11	symmetrical	
15A	11♂ 4♀	4	3	1
16A	12♂ 4♀	13	7	6
17A	14♂ 4♀	1		
18A	14♂ 4♀	2	1	1
19A	15♂ 4♀	1		
20A	22♂ 4♀	1		
21A	22♂ 4♀	1		
22A	23♂ 4♀	1		
23A	24♂ 4♀	1		
24A	24♂ 4♀	1		
25A	26♂ 4♀	1		
26A	13♂ 5♀	1		
27A	16♂ 5♀	1		
28A	24♂ 5♀	1		
29A	24♂ 5♀	1		
30A	27♂ 5♀	1		
31A	31♂ 6♀	1		

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arising at that node are unequal; and from that condition it is only one step to the complete suppression of one branch, and when this happens the dichasium passes over into the cincinnus (or uniparous scorpioid cyme).

Studying the inflorescences of *B. semperflorens* gave the impression that the two branches of the same dichotomy form unequal angles with the main axis and that the stronger branch frequently forms a more acute angle than the weaker one with the extension of the axis (or with the pedicel of the staminate flower at that node). The securing of data on

(or with the pedicel of the staminate flower at that node); this is illustrated in the primary dichotomy of figures 6 and 6A, 7 and 7A, 8 and 8A, 9 and 9A, 21 and 21A, 22 and 22A. In seven instances the reverse was true; the weaker branch formed a more acute angle, as shown in figures 20 and 20A. In one case the branches were unequal as described above, but both formed the same angle. While these figures are too small to be completely convincing, they are at least suggestive. This feature is a further foreshadowing of the cincinnus within the dichasium. In figures 1A to 31A the angles at successive dichot-

omies have been kept proportionally similar to those of the actual photographs, the limitations of the diagrams often making exact reproduction impossible. The proportions in each case have been kept unchanged.

Records were kept and photographs taken of the arrangement of staminate and pistillate flowers in 106 inflorescences of *Begonia semperflora* *erfordia*. The staminate flowers in each inflorescence mature before the pistillate, though there may be overlapping in anthesis of the two types. In figure 32, for example, six staminate flowers had already dropped off, four remained associated with the four pistillate flowers when the photograph was taken. Both types are apparently functional simultaneously.

Among the 106 above-mentioned inflorescences, with varying numbers of staminate and pistillate flowers, 31 different patterns occurred. These are illustrated in figures 1-31, in each of which, with a few exceptions, only the pistillate flowers remain. Figures 1A-31A are diagrams of these same inflorescences, in which each staminate flower is represented in position by a "♂" sign, and each pistillate flower by a "♀" sign. In table 2 the number of times that each of these types occurred is given. From this table it is obvious that among the 106 inflorescences three had four staminate and two pistillate flowers, and in each of the three these flowers were arranged as in figure 1A or its mirror image; three inflorescences were found with five staminate and two pistillate flowers, and these all had the flowers arranged as in figure 2A. Since this inflorescence is symmetrical, from the standpoint of arrangement of staminate and pistillate flowers (disregarding internodal lengths), its mirror image would be identical with the figure. All the diagrams were made looking down upon the inflorescences from above, as they grew on the plants. Table 2, column 3, shows how frequently each type or its mirror image occurred, the mirror image arrangements occurring just about as frequently as those in the diagrams, as indicated in columns 4 and 5. Figure 6A actually was found 4 times, its mirror image 4; consequently it is given as occurring 8 times; figure 13A was found 6 times, its mirror image 5; figure 16A was observed 7 times, its mirror image 6, etc. The two right-hand columns of table 2 show how frequently the figure of the diagram occurred and how frequently its mirror image was found in each instance in which more than one inflorescence of a type was studied. Those marked symmetrical have the mirror image identical with the diagram, since they are bilaterally symmetrical. The occurrence of these diagrams and of their mirror images in approximately equal numbers (35 and 33) would be expected.

Only one or two inflorescences occurred, among those recorded, having more than 12 staminate flowers and 4-6 pistillate flowers (fig. 17A-31A). However, the types represented by figures 1A-16A occurred frequently enough to be of significance in one way at least. For instance, eleven inflorescences occurred with 10 staminate and 4 pistillate flowers; every one

of these eleven inflorescences had those flowers arranged as in figure 13A or the mirror image of that figure. There were nine inflorescences which had 9 staminate and 3 pistillate flowers, all of which had those flowers as in figure 8A or its mirror image. Eight inflorescences were found with 8 staminate and 3 pistillate flowers, all arranged as in figure 7A (or its mirror image). While these numbers are not large, the fact that all the inflorescences had the flowers arranged in identical patterns makes these patterns significant. Inflorescences with larger numbers of flowers show several patterns in many cases, as is obvious from figures 14A and 15A, 17A and 18A, 20A and 21A, 23A and 24A, 28A and 29A, though even in these the differences are often only minor.

This relative constancy of pattern was unexpected. The inflorescences, to be sure, are built on the general plan of the dichasium passing over into the cincinnus. However, even this general scheme allows for much greater flexibility than is revealed in these 106 inflorescences. The minimum number of patterns possible among the 90 inflorescences of figures 1A to 16A with the numbers of staminate and pistillate flowers represented is 15, and 16 actually occurred. The maximum number possible would be 90. Such regularity and fixity seem striking. Figures 17A-31A reveal greater variability, and the deduction would be that larger and more complex inflorescences are less stable in pattern. A study of these types shows that they do not differ markedly from each other, and usually only one or two inflorescences with each combination of staminate and pistillate flowers occurred, so that these numbers are too small to be convincing.

Disregarding internodal lengths, figures 2A, 4A, 5A, 10A, 12A, and 14A are bilaterally symmetrical in the arrangement of staminate and pistillate flowers. Each of these shows a balanced inflorescence type. In figures 10A and 14A both branches are bilaterally symmetrical within themselves, while figures 8A, 11A, 13A, 16A, 18A, 20A, 21A, 25A, 26A, 27A, 28A, and 31A illustrate a similar symmetry within either the right or left branch of the inflorescence. This symmetry is relatively simple in a case like the left branch of figure 28A, and less so in the left branch of figure 31A, which forks again but nevertheless maintains internal bilateral balance. Of the thirty-one inflorescence types illustrated, eighteen show pronounced bilateral symmetry to a greater or less extent. Unbranched inflorescences sometimes occur in this species.

It is further obvious from figures 1A-31A and table 2 that the ratio of staminate to pistillate flowers within the inflorescence is by no means constant. Figure 1A, for instance, has a ratio of 2 staminate flowers to 1 pistillate; in figure 3A the ratio is 3 to 1, in 5A it is $4\frac{1}{2}$ to 1. Among the inflorescences with four pistillate flowers the ratio of staminate to pistillate varied from $1\frac{3}{4}$ to 1 in figure 10A to $6\frac{1}{2}$ to 1 in figure 25A. This is the highest proportion of staminate to pistillate flowers that occurred in any of the inflorescences shown. Inflorescences having five pistillate flowers showed ratios of $2\frac{3}{4}$ to 1 in figure 28A

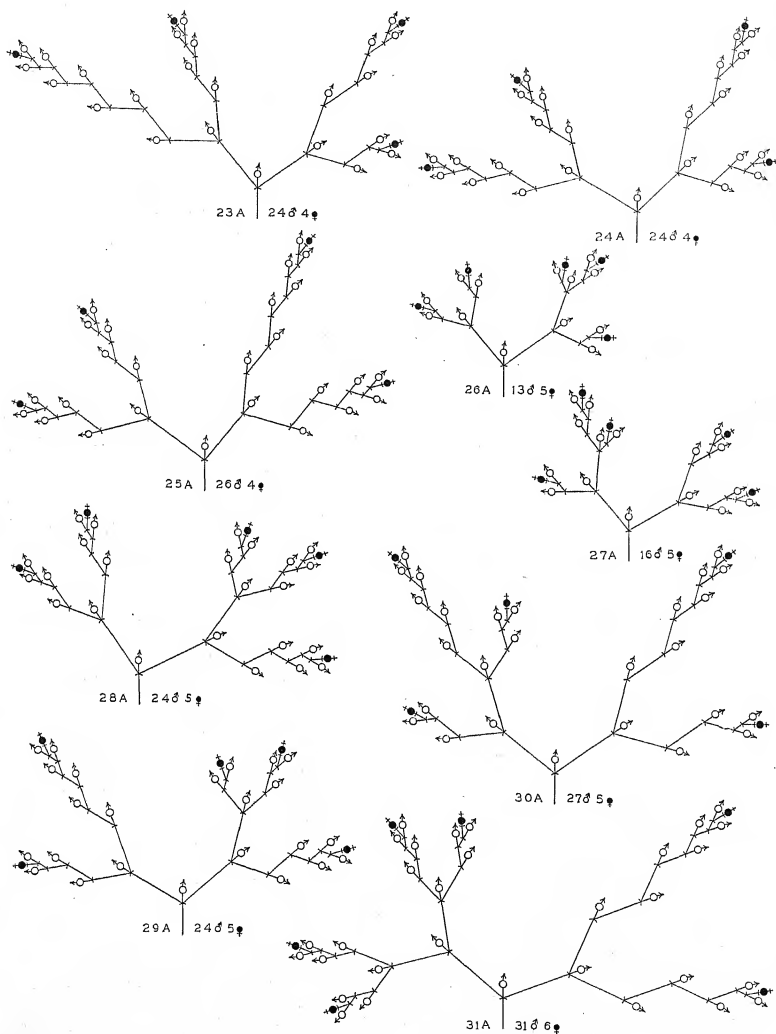


Fig. 23A-31A. Diagrams of inflorescences shown in fig. 23-31, each staminate flower shown in position by a "♂", each pistillate flower by a "♀". The numbers at the right of each figure indicate the number of staminate and pistillate flowers in each inflorescence.

to $5\frac{1}{2}$ to 1 in figure 30A. While the larger inflorescences tend to appear after the plants have been in flower for some time, they do not necessarily show a higher ratio of staminate to pistillate flowers, as may be deduced from figures 26A and 27A. In general the first inflorescences on a plant tend to have fewer flowers, the larger ones appearing later, though small branches of an older plant commonly also have inflorescences with a small number of flowers. All the types illustrated in figures 1A–31A did not occur on one plant, but a single plant in the course of its flowering shows a considerable range of inflorescence types.

EFFECT OF SOIL FERTILITY ON SEXUAL EXPRESSION.—To gain some insight into the possible causes of the production of staminate and pistillate flowers, plants of four different varieties of *Begonia semperflorens* were raised from seeds, all of which were planted at the same time. The seedlings of all varieties were transferred into boxes, and when the plants were large enough to handle, they were divided into lots. The first lot, consisting of fifty-three plants, of all four varieties, was transferred into three-inch pots containing unwashed coarse brown sand. No leaves were used in the bottom of these pots. The second lot consisted of sixty plants of the same four varieties, and these were transplanted first into four-inch pots and later into six-inch pots. These were grown in rich loam consisting of five parts of clay loam, one part of leaf mould, and one-third of a part of sand, and to this bone meal, sheep manure, and vigor were added. These plants grew vigorously and became thrifty. The plants in sand and in loam began flowering from the second week in July on; some of those in sand commenced flowering as early as those in the loam; in general, however, those in the sand began somewhat later, and consequently those in loam were exposed to a longer day length during part of their flowering period than those in sand. All the flowers that were visible were counted. This includes those that matured and opened, and it includes many that never passed beyond the bud stage. Since the flowers may drop off in all stages of development, it is altogether probable that many flower primordia form and never become visible to the naked eye, but shrivel and die before they have developed far enough to become noticeable. This may well be true especially of pistillate flowers, since they are the last to develop in an inflorescence. However, when a plant forms the normal staminate flowers of an inflorescence, and the pistillate flowers of that inflorescence fail to become visible, and then additional inflorescences with normal staminate flowers are produced in which all or some of the expected pistillate flowers also fail to mature, it seems justifiable to say that femaleness, as expressed by the production of pistillate flowers, is suppressed, to some extent at least. The 53 plants grown in sand produced an average of approximately seven inflorescences per plant. The largest number was nineteen, two had eighteen, and several had only one. All of these began by forming staminate flowers.

These inflorescences develop on a plant in sequence, not simultaneously, and the first flowers of each are staminate; they form whether the preceding inflorescences have developed pistillate flowers or not.

Records of the flowering of these 113 plants were kept, and the flowers and inflorescences marked. The totals were then tabulated for each month of flowering; the last records were made on December 10, though many plants had previously ceased flowering. Records of partial inflorescences or of inflorescences that had obviously not completed their flowering were not included, since the staminate flowers normally are produced before the pistillate.

The difference in general appearance, height, branching, and flowering between plants grown in the clay loam and those grown in sand is shown in figures 34 and 35. Figure 34 is a photograph of two plants of *B. semperflorens erfordia* raised from seeds planted on the same day. The one on the left is in sand, the one on the right in loam. These plants were grown side by side in the greenhouse, are both the same age, and received similar treatment except for soil nutrition. Figure 35 is also of two plants, again of the same variety, *B. semperflorens prima donna*, both grown from seeds planted simultaneously, the one at the left in sand, the other in loam. A 30-centimeter rule gives the scale in figures 34 and 35. The dwarfed

TABLE 3. Ratio of staminate to pistillate flowers in four varieties of *Begonia semperflorens* grown in sand and in clay loam.

<i>Begonia</i>	♂ flowers	♀ flowers	Ratio	No. of plants
<i>B. semperflorens dwarf vernon</i>				
Sand	329	29	11.34:1	17
Clay loam	811	113	7.18:1	18
<i>B. semperflorens erfordia</i>				
Sand	813	224	3.63:1	15
Clay loam	5775	2149	2.69:1	16
<i>B. semperflorens prima donna</i>				
Sand	150	39	3.85:1	11
Clay loam	2196	863	2.54:1	13
<i>B. semperflorens luminosa</i>				
Sand	163	33	4.94:1	10
Clay loam	1345	451	2.98:1	13
Totals				
Sand	1455	325	4.48:1	53
Clay loam	10127	3576	2.83:1	60
	11582	3901		113
	15,483 flowers			

plants in sand flowered in spite of their small size, as indicated in figure 33, a photograph of a plant of *B. semperflorens dwarf vernon* in a three-inch pot, with a few leaves and a staminate flower. The bud of another staminate flower, behind the one that is open, is barely visible. A centimeter scale is shown below the pot.

The results of observations on 113 plants, including a total of 15,483 flowers, are given in table 3. The number of flowers, both staminate and pistillate, was



Fig. 32-35.—Fig. 32. Inflorescence of *B. semperflorens erfordia*, with four staminate flowers above and four pistillate flowers below. Six additional staminate flowers had completed anthesis and fallen when the photograph was taken.—Fig. 33. Plant of *B. semperflorens dwarf vernon* grown in sand in a 3-inch pot, showing several leaves and a staminate flower. A bud of another staminate flower is also present. The scale is in centimeters.—Fig. 34. Two plants of *B. semperflorens erfordia*, both of the same age, the one on the left grown in sand, the one on the right in rich loam. A 30-centimeter rule is shown below.—Fig. 35. Two plants of *B. semperflorens prima donna*, both of the same age, the one on the left grown in sand, the one on the right in rich loam. A 30-centimeter rule is shown below.

much greater on plants grown in loam than on those in sand. In each of the four varieties, however, the ratio of staminate to pistillate flowers was higher in plants grown in sand than in those grown in loam. The ratio of staminate to pistillate flowers on all varieties added together is 4.48 staminate flowers to 1 pistillate in sand and 2.88 staminate to 1 pistillate in loam. Since the ratios in all four varieties are similar, this difference seems to be significant. It may be concluded then that plants growing under rich soil conditions develop a relatively larger number of pistillate flowers, those growing in poor soil (sand) have a larger proportion of staminate flowers. As pointed out above, the present study does not show

whether this difference is due to a complete failure of pistillate flower primordia to appear, whether young primordia are formed but do not develop further, whether minute buds, which do not show noticeably beyond the bracts, fail to complete their development, or whether the inflorescence patterns of plants grown in sand differ from those in rich loam. It is not at all inconceivable that one, two, three, or all four of these may occur. In all probability none of them, not even the last, can be distinctly separated from the others.

DISCUSSION AND CONCLUSIONS.—Whatever the organic basis for the separation of staminate and pistillate flowers may be, the data given above indicate

that this separation is constant, as biological phenomena go, in the plants studied. As was pointed out above, only a single flower with apparently functional stamens and pistils was found, though others which were intermediate in other ways also occurred. It is possible to conceive of this definite and regular separation of the two kinds of flowers as a result of chemical or nutritive or general metabolic differences. According to Pastrana (1932), it is accompanied by a chromosomal difference in *Begonia Schmidtiana*. The critical stages in the actual loss of a chromosome in the formation of a staminate flower have not been figured, however. And the report of fairly large and varying chromosome numbers in numerous species of *Begonia* by Heitz (1927) would make the establishing of this procedure for the genus generally especially difficult. Mereminski (1936) found no difference in the number of chromosomes in the flowers of *B. incana*. Whatever the cause or causes of this separation of the microsporophylls and megasporophylls may be, the relative infrequency of perfect flowers in the material studied indicates that the factors promoting it are effective. Since staminate and pistillate flowers differ not merely in the presence or absence of stamens and pistils, but also in their position in the inflorescence and in the number of perianth parts and bracts, *Begonia semperflorens* is obviously favorable material in which to study the separation of the two kinds of flowers.

The development of stronger and weaker branches, which at final false dichotomies bear respectively a larger and smaller number of flowers, is a distinct foreshadowing of the failure of one branch to develop at all; and this happens at those nodes in the inflorescence which are terminated by a staminate flower and bear but one branch and not two. Growth substance differences might be expected in these weaker and stronger branches, but they would be only a step in explaining the occurrence of these two kinds of branches, a feature which is rather general in cymose inflorescences. The more acute angle that the stronger branch of a dichotomy may form with the extension of the axis of the inflorescence, or with the pedicel of the staminate flower, tempts the suggestion of functional hypertrophy, and in freely branching systems such as these inflorescences growth against pressure may well be of significance; in other cases in the plant kingdom this might be more difficult to establish. The easier transport of nutrients to a branch more nearly erect and the more favorable location of branches in other ways are further possibilities. Harper (1926) has similarly stressed growth against pressure in the determination of form in *Dictyosteleum*.

The inflorescences shown in figures 2A, 4A, 5A, 10A, 12A, and 14A are essentially bilaterally symmetrical, and consequently they achieve a definite balance, both in their pattern and in reality. That this is of significance more especially in the earlier stages of development seems not merely possible but probable. There are inflorescences, such as that illustrated in

figure 15A, which are not bilaterally symmetrical either in their entirety or in any of the branches of which they are constituted. How much this asymmetry is compensated for by a difference in other factors, such as the angle of branching, for instance, is unknown. In summary, eighteen of the thirty-one inflorescence types illustrated—and they were taken at random—show bilateral symmetry, when considered as a unit, or in one or more of their branches. Asymmetries may be equalized, from the standpoints of balance or of nutrition, by compensating factors, such as angles of branching, internodal lengths, and branch diameters.

Aspects of symmetry may be significant in determining the arrangement of flowers within the inflorescence. But they can operate, apparently, only within limits, which differ greatly for different plants. In the variety of *B. semperflorens* analyzed here those limits are defined by the dichasium, passing over sooner or later into the cincinnus. Bilateral symmetry is frequently established, and even when it is not maintained, asymmetries may be counterbalanced by other features, such as length of internode and angles of branching.

Since the staminate flowers of an inflorescence mature before the pistillate, the inflorescences and plants may be spoken of as dichogamous. As shown in figure 32, however, not all the staminate flowers necessarily drop off before the stigmas of the pistillate are receptive. The inflorescences, and to a relatively smaller extent the entire plants, are protandrous. Each inflorescence goes through a period in which it is functionally staminate, then later may have both staminate and pistillate flowers in anthesis, and finally the staminate flowers all drop off, leaving only the pistillate open and receptive. The inflorescences, and to a less extent the entire plants, may illustrate incomplete dichogamy (Stout, 1928), or if all the staminate flowers drop off before the pistillate open, as is frequently the case, the dichogamy within the inflorescence may be complete. Complete dichogamy within a single plant does not normally occur in the varieties of *Begonia semperflorens* studied.

From table 2 and figures 1A–31A it is obvious that the ratio of staminate to pistillate flowers within the individual inflorescence is subject to considerable variation. Table 3 shows that the same statement applies to single plants; it indicates further that the development of staminate and pistillate flowers on a plant is governed, to an extent at least, by nutrition. The more richly nourished plants bear a greater number of flowers and also a larger proportion of pistillate flowers. Changing the ratio or nature of the flowers by varying the environmental conditions has been done repeatedly, and Schaffner (1922) was able to vary the expression of *Arisaema triphyllum* from carpellate to staminate and back to carpellate by changing the environment; fertile soil and moisture induced pistillate flower formation. In *Veratrum* abundant nourishment induced the production of

perfect flowers, while starved branches bore flowers that were functionally staminate (Lewitsky, 1925). Talley (1934) found that in pistillate plants of hemp nitrogen is relatively more abundant than in staminate, though the latter have higher percentages of carbohydrates. Although Jack-in-the-pulpit and hemp are more commonly considered dioecious, and *Begonia semperflorens* is monoecious, this difference is one in extent rather than in kind, since both *Arisaema triphyllum* and *Cannabis* may have stamens and pistils on the same plant. The variability in the number of flowers in the inflorescence of *B. semperflorens* is in rather striking contrast to the constancy found by Irmscher (1924) in *B. Wallichiana*.

In *B. semperflorens* the sexual expression, as manifested by the production of staminate and pistillate flowers in varying proportions, is obviously correlated with metabolic differences. Within definite limits these differences affect the formation of staminate and pistillate flowers, and therefore indirectly the formation of male and female gametophytes. The term sexual expression is used here in the wider sense, to include the two types of flowers, not in the more limited connotation, referring to the gametes or gametophytes. The metabolic differences of the sporophyte are reflected, at least, in the development of the succeeding haploid generation. While the environment may be of definite significance, it operates only within certain limits, which vary for different genera and which are fairly constant within the species or variety from one generation to the next. These limits, therefore, are inherited. Well nourished plants of *Arisaema triphyllum* produce practically all pistillate flowers, while those of *Begonia semperflorens* bear merely a higher proportion of pistillate flowers, but have staminate flowers as well. The part that heredity plays in determining the limits within which the sexual expression may be modified by the environment is markedly important. In some plants these limits are so narrow that they tend to disappear, and environment seems to have a minimum effect on the sexual expression. In other forms, such as those discussed above, the hereditary impositions are much less rigid, and external environment is significant in determining the sexual expression.

In haploid generations similar conditions obtain. Certain genera, of bryophytes such as species of *Sphaerocarpos* and *Marchantia*, are relatively definite and rigid in their sexual expression, while others, such as the gametophytes of some of the Polypodiaceae and of the much discussed *Equisetum*, vary within wider limits in this expression.

Sex may be considered, then, to be determined in part by the inherited limits within which its expression may vary. In some plants these limits are rigid and narrow and allow of little variation, while in others they are wider. In the latter sexual expression is determined partly at least by external factors. This is true of *Begonia semperflorens*.

In becoming more staminate or more pistillate this species illustrates a stage in the transition from monoe-

cism to dioecism—perhaps the very first step in this direction. In the occurrence of only occasional flowers bearing both stamens and pistils the last vestige of the probable change from the perfect to the monoecious flower is also observed. If imperfect flowers have been derived from perfect ones, and dioecious plants from monoecious types, as is commonly supposed, then *Begonia semperflorens* offers an occasional glimpse of the former perfect-flowered condition, in the rare occurrence of such flowers; it also illustrates, perhaps, the first step in the change from the monoecious to the dioecious condition, since certain plants show a larger proportion of staminate flowers, others relatively more pistillate flowers.

SUMMARY

In four varieties of *Begonia semperflorens* studied, staminate and pistillate flowers normally occur in the same inflorescence, but flowers with both stamens and pistils are very infrequent. Flowers intermediate in other respects are found occasionally.

The typical inflorescence is a dichasium passing over sooner or later into a cincinnus. The final forkings within the inflorescence consist of a longer internode which usually supports more flowers and a shorter one which supports fewer. This foreshadows the condition in the cincinnus, in which one branch (the shorter) fails to develop, and hence bears no flowers.

In fourteen of twenty-two cases in which measurements were made the stronger branch of two at the same node in an inflorescence formed a more acute angle than the weaker branch with the extension of the main axis or with the pedicel of the staminate flower at that node.

Incomplete dichogamy occurs in the individual inflorescences and in the single plants. Dichogamy within the inflorescence is complete in some cases.

Varying ratios of staminate to pistillate flowers occur in the different inflorescences on the same as well as on different plants.

The pattern of branching and of distribution of staminate and pistillate flowers within the inflorescence shows little variation for any definite ratio of staminate to pistillate flowers.

Within the hereditary limits of possibility in the inflorescence, symmetry, especially of the bilateral type, is frequently attained, which establishes balance. Apparent asymmetries may be compensated by other factors, such as angles of branching or internodal lengths.

Well nourished plants grown in clay loam produced staminate and pistillate flowers in the ratio of 2.83:1; poorly nourished plants grown in sand produced staminate and pistillate flowers in the ratio of 4.48:1. Hence the well nourished plants in loam were more strongly pistillate in their expression, those in sand more strongly staminate.

It is suggested that sex is determined in part by the inherited limits within which its expression may vary. If these limits are narrow, environment has

little effect on its expression; if these limits are wide, the environment is of marked importance. In *Begonia semperflorens* the environment is significant.

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STUDIES ON CYTOLOGY AND RESISTANCE TO LEAF RUST OF SOME INTERSPECIFIC AND INTERGENERIC HYBRIDS OF WHEAT¹

A. T. Guard

STUDIES WERE made of crosses of *Triticum vulgare*, Chinese (C.I. 6223),² with the following species: *T. dicoccum*, Vernal (S.D. 293),³ *Secale cereale* Abbruzzes, and *S. montanum*. The chromosome number of the parental species as reported by previous workers and checked by the writer are as follows: *T. vulgare*, twenty-one; *T. dicoccum*, fourteen; *S. cereale* and *S. montanum*, seven. Reduction divisions in pollen mother cells of all species used proceeded normally with neither lagging nor univalent chromosomes. The variety, Chinese, of *T. vulgare* was very susceptible to leaf rust in the seedling stage, and all other species were highly resistant.

METHODS.—Chromosomes were studied during reduction divisions of the pollen mother cells by means of both temporary smear mounts and permanent slides. Permanent smear mounts were made by McClintock's method (1929). Anthers for sectioning were killed either in Karpechenko's modification of Nawaschin's solution or in Darlington's modification of Flemming's. Sections were cut fifteen microns in thickness and stained either in Newton's gentian-violet or in Heidenhain's iron-alum haematoxylin. Sections stained in the latter solution were destained in picric acid according to the method of Tuan (1930).

Plants were grown in the greenhouse and were inoculated in the seedling stage with *Puccinia tritica* physiologic race 65. The rust reaction of each individual plant was observed. Heads of the F₁ and F₂ generations were bagged to prevent cross pollination, and other generations were allowed to mature without bagging.

HYBRIDS BETWEEN CHINESE WHEAT AND VERNAL EMMER.—The F₁ plants grew vigorously and produced well developed spikes. A few plants were completely sterile, whereas all others produced from three

to ten seeds per spike. No plant approached complete fertility. Seed from the F₁ hybrids were of medium size and plump. The spikes were characterized by the short incurved awns of the seed parent and by the keeled glumes and brittle rachis of the pollen parent (fig. 1).



Fig. 1. Heads from: Vernal emmer (left), F₁ hybrid (center), Chinese wheat (right).

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² (C.I. 6223) indicates the accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations), United States Department of Agriculture.

³ (S.D. 293) indicates the identifying number given to this line by the South Dakota Agricultural Experiment Station, Brookings, South Dakota.

Pollen mother cells of the F₁ hybrids showed fourteen bivalents and seven univalents at meiosis I. The univalents were still scattered in the cytoplasm (fig. 2) when the bivalents had arranged themselves at the equatorial plate. This delay of univalents was very brief, and no chromosomes were excluded from the nucleus during meiosis.

The F_1 hybrids showed an X reaction to physiologic race 65 which varied from a high susceptibility to almost complete resistance. Johnston and Mains (1932) reported the occurrence of this X type of reaction very common on F_1 hybrids when resistant and susceptible wheat varieties were crossed.

Twenty-four lines, selected in the third generation, were studied in the fourth and fifth generations. The haploid number of chromosomes in seven of these lines was fourteen, and in the remaining seventeen lines the haploid number was twenty-one. No univalents were present in any of these lines, and lagging of chromosomes was rarely observed. The pollen appeared normal.

All lines possessing fourteen chromosomes were as rust-resistant as the pollen parent. Of the seventeen lines with twenty-one chromosomes, as their haploid number, eleven were resistant and six were highly susceptible. This apparently larger number of resistant lines was secured from a more intensive study directed to that phase of the problem.

In crosses of common and emmer wheat Sax (1923) secured no rust-resistant segregates with twenty-one chromosomes. Tochinal and Kihara (1927) concluded that "a rust-resistant wheat-strain will hardly be obtained from crossing emmer and *vulgare* wheat, though it may not be entirely impossible." In a cross between Yaroslav emmer and Marquis, McFadden (1930) secured several twenty-one chromosome lines which were very resistant to leaf rust. Notable among these were varieties Hope and H-44.

HYBRIDS BETWEEN CHINESE WHEAT AND ABRUZZES RYE.—A study was made of one line from a cross between *T. vulgare*, Chinese (6223), a spring wheat, and an inbred self-fertile strain of Abruzzes winter rye. Seed of the F_2 generation of this hybrid was made available through the courtesy of Dr. E. B. Mains. Heads of the F_1 and F_2 plants were bagged to prevent cross fertilization. All material studied was the progeny of a single F_2 plant.

Plants of the F_2 and subsequent generations grew vigorously and developed large spikes. They were all of spring habit. Their fertility varied from 5 to 75 per cent. Lebedeff (1934) reported a fertility of 38 per cent in wheat-rye amphidiploids studied by him. Variations in fertility observed seemed to be correlated more with environment than with genetic differences of the progeny within the line. Fertility was greater in the greenhouse than in field-grown plants. Examination of the flowers at the time of blooming indicated that the retarded germination of the pollen grains and the rather ephemeral nature of the pistil probably had much to do with this sterility. The pollen tubes did not begin to develop until about twenty-four hours after the glumes first opened, and before this time the pistil frequently had begun to wither. In the hot dry atmosphere which often prevailed in the field at the time of heading, this condition was doubtless aggravated.

Twenty-eight bivalent chromosomes were counted in the pollen mother cell of this hybrid (fig. 3). No

univalent chromosomes were present. Lagging chromosomes were frequently observed, but only in rare instances was any chromosome excluded from the nucleus. The number of lagging chromosomes ranged from none to four and was most frequently two. Only the usual four microspores were formed from one pollen mother cell, but occasionally nuclear fragments were observed in the cytoplasm of the microspores.

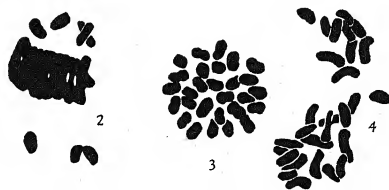


Fig. 2-4. $\times 1500$.—Fig. 2 (left). Pollen mother cell from F_1 hybrid between Vernal emmer and Chinese Wheat, showing seven lagging chromosomes.—Fig. 3 (center). Chromosomes of a pollen mother cell from the amphidiploid wheat-rye hybrid.—Fig. 4 (right). Pollen mother cell from the F_1 hybrid of a cross between Chinese Wheat and *Secale montanum* showing twenty-eight univalent chromosomes.

The presence of twenty-eight bivalent chromosomes, the high degree of regularity, and the fertility of this hybrid all indicate that it is an amphidiploid, having in the somatic cells, two complete sets of chromosomes from each parent. Such amphidiploids have been reported by Lebedeff (1934) and Lewitsky and Benetzkaia (1931).

This amphidiploid strain resembles those reported by Lewitsky and Benetzkaia (1931) in the high degree of fertility and in the presence of twenty-eight chromosome pairs in the pollen mother cells. It differed from the amphidiploids studied by these authors in that no additional nuclei were observed at the tetrad stage, the cell dimensions of the microspores were very uniform, isolated univalents were observed only very rarely, and in no instance were chromosomal fragments observed in addition to the twenty-eight bivalents.

This hybrid was highly resistant to leaf rust.

HYBRIDS OF CHINESE WHEAT AND SECALE MONTANUM.—Fifteen F_1 plants of Chinese wheat *T. vulgare*, \times *S. montanum* were studied. These two species crossed readily. Although the endosperm was somewhat shrunken, the seed germinated well and produced vigorous plants. All F_1 plants were sterile.

Stamens of three plants were abortive at a very early stage of development. The floral organs of all others developed to normal size, but the anthers failed to dehiscence. Examination of pollen from twelve plants showed all grains to be nearly devoid of protoplasm and obviously non-viable.

No pairing of chromosomes was observed prior to diakinesis. At this stage one bivalent was rarely

observed. There was no clear-cut metaphase in meiosis I. The twenty-eight univalents (fig. 4) moved to the poles without splitting. Tripolar spindles were frequently observed. Florell (1931) reported a similar condition in wheat-rye hybrids. Meiosis II was accomplished with much more regularity. There were lagging chromosomes, but usually nearly all chromosomes formed a definite equatorial plate. The chromosomes split at this stage. The microspores varied greatly in size, being both larger and smaller than normal. Five to seven microspores, instead of the usual four, were frequently formed from one mother cell. Two nuclei were observed in some microspores. The extra nucleus, however, was obviously one which resulted from a lagging chromosome group rather than from the first division of the microspore nucleus. No pollen cells were found which contained the three nuclei characteristic of the pollen of the parent species at the time of shedding. Anthers of these hybrids were not observed to dehisce.

The F_1 plants were highly resistant to leaf rust.

SUMMARY

In a study of twenty-four lines from crosses between Chinese wheat and Vernal emmer, seven lines had a haploid chromosome number of fourteen, while in seventeen lines the haploid number was twenty-one. All lines with the lower chromosome number were resistant to leaf rust, physiologic race 65, in the seedling stage. Six of the lines with twenty-one chromosomes were susceptible and eleven were resistant.

The wheat-rye hybrid studied was an amphidiploid. Meiosis showed only minor irregularities. Fertility varied from 5 to 75 per cent, depending largely on environmental conditions, particularly at flowering time. This line was very resistant to physiologic race 65 of leaf rust.

Fifteen F_1 hybrids between Chinese wheat and *Secale montanum* were sterile. Meiosis was very irregular. No viable pollen was produced. These plants were very resistant to leaf rust, physiologic race 65.

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ANATOMICAL EVIDENCE FOR THE MENYANTHACEAE¹

Alton A. Lindsey

For a century opinion has been divided regarding the systematic rank of the peculiar group of aquatic and palustrine genera including the Buck-bean (*Menyanthes trifoliata* L.). These five genera (*Nephrrophyllum*, *Menyanthes*, *Villarsia*, *Nymphoides*, and *Liparophyllum*) have been assigned to the Gentianaceae by most authors, but the group was considered a separate family by Don (1838), Britton (1897), and Wettstein (1935). In the arrangement of Gilg (1895) for *Die Natürlichen Pflanzenfamilien*, the Gentianaceae is divided into two sub-families, the five

genera in question constituting the Menyanthoideae. Thus defined, the family includes about sixty-four genera and over eight hundred species.

It is now recognized that the characters derived from the internal anatomy of the flower, especially its phylogenetically conservative vascular skeleton, are of fundamental significance for natural classification. The writer's investigation of the floral anatomy of eighty-six species of the Gentianaceae, representing forty-nine genera, provided a basis for comparison of the two "sub-families" from this viewpoint.

Four genera of the Menyanthoideae were studied, the rare New Zealand genus *Liparophyllum* being omitted because the material obtained proved unsuitable for anatomical work. Six species of the group were used; two of the genera studied are monotypic. The method followed in most instances was to embed from a butyl alcohol series, section and

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mount serially, and stain with erythrosin and crystal violet.

DESCRIPTIONS. — *Gentianaceae*. — The anatomical evidence bearing on the affinity of the Menyanthaceae and Gentianaceae must necessarily be derived from a study of both groups. Therefore, the following summary of the floral anatomy of the Gentianaceae is given for purposes of comparison with the descriptions of menyanthaceous genera presented later.

The fundamental anatomy of the gentianaceous flower may be illustrated by the condition in *Centaurium*, a genus of the sub-family Gentianoideae. The five traces to the calyx arise as a whorl, leaving gaps in the stele just above their points of origin (fig. 1). The calyx traces pass outward from the stele and each splits into three branches, a midrib and two laterals. Usually at a slightly higher level the calyx tube becomes free from the receptacle and encircles it, while the corolla traces are leaving the central stele, followed by the traces to the stamens (fig. 2). These successive whorls occupy successively alternating radii, so that the stamen traces occupy the same radii as the calyx traces. The corolla traces each divide in three at a higher level (fig. 3), and the gaps left in the stele by the departure of the stamen traces close to produce a hollow cylinder. The latter typically breaks into six traces; a dorsal with two ventrals, one on each side, supply each of the two carpels (fig. 4). The base of the locule often first appears as a pair of cavities separated by a shallow septum representing the fused bases of the placentae, which separate higher in the ovary to form two protruding placentae (fig. 5).

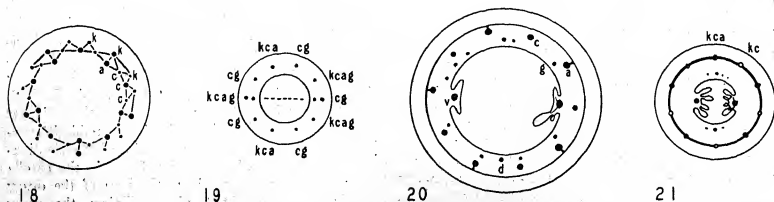
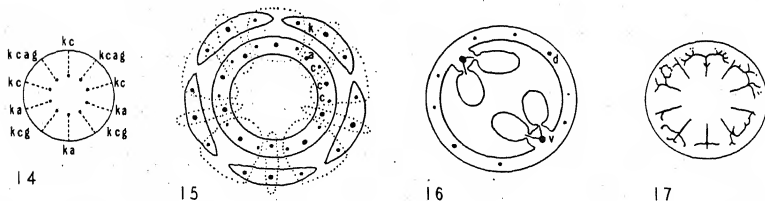
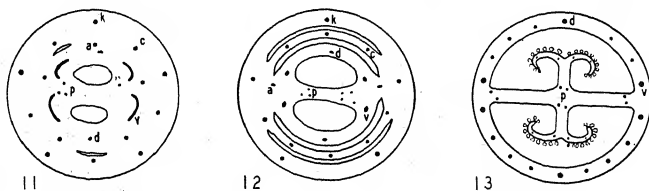
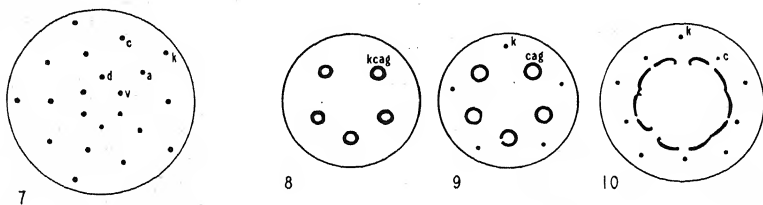
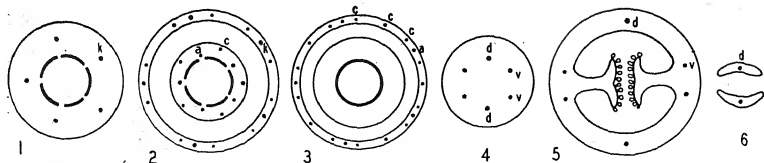
The type of placentation is extremely variable in the Gentianaceae. In some genera the inner lining of the locule bears the ovules along two areas more or less restricted to the region of the ventrals. In this case there may be no indication whatever of the protrusion of the placentae into the cavity of the ovary. When protruding placentae are present, they may be supplied by strong or weak branches of the ventral, or they may lack lignified vascular tissue, parenchyma tissue resembling phloem parenchyma serving for conduction. Usually the dorsals alone extend into the stigma (fig. 6).

The fundamental vascular pattern illustrated by *Centaurium*, shown in ground plan in figure 7, is found in several genera of the Gentianaceae. However, this simple arrangement is considerably modified by fusion in the more advanced genera. The calyx laterals, instead of being fused with their midrib trace at its origin, often arise fused with the laterals of the adjacent sepals. In a few species each such double lateral is adherent with the corolla trace lying in the same radius. Cohesion of the carpel traces commonly occurs, each ventral merging with the adjacent one of the other carpel. This results in four main traces to the ovary rather than the usual six. In the tribe Exacinae, placental bundles exist as well developed branches of the ventrals, and these placentals may be fused together in various ways in the bilocular ovary.

Macrocarpaea represents an extreme fusion type in the Gentianoideae. Below the receptacle is a dictyostele of five discrete concentric bundles (fig. 8). Each comprises a hollow cylinder of phloem surrounding a xylem cylinder which in turn encloses a central parenchymatous core. At this level each bundle includes vascular tissue destined for the calyx, corolla, androecium, and gynoecium. The first traces given off are the five calyx traces (fig. 9), each leaving a gap in the fusion bundle from which it is derived. Above the gap each fusion-bundle (bearing corolla, stamen, and carpel vascular supply) is again concentric, forming a "pseudostele" of the same nature as those described by Woodson (1936) and Wilson (1937). Soon, however, each bundle splits along its inner side and spreads laterally by progressive separation of the edges as higher levels are reached. Adjacent bundles fuse laterally across the calyx-trace radii to form a siphonostele which is dissected slightly higher by the gaps of the departing corolla traces (fig. 10). With the differentiation of the two dorsals and four ventrals, minor branches of the ventrals (placentals, fig. 11, p.) move inward to supply the placentae and the placental septum. The five stamen traces show a two-fifths spiral arrangement in their order of leaving the stele. In the Gentianoideae, sets of floral organs which appear whorled externally often clearly show other "phyllotactic" arrangements in the origin of the traces, having the decussately opposite pattern in four-merous flowers and the two-fifths spiral arrangement of traces in five-merous species. In *Macrocarpaea* the heavy calyx tube, the corolla, and the ovary remain fused together for a considerable distance upward on the radii of the two stamen traces which leave the stele last (fig. 12). For a considerable distance upward in the ovary (fig. 13) the placentae are fused, producing a bilocular ovary.

The peculiar concentric fusion-bundles were found in four genera (*Prepusa*, *Schultesia*, *Chelonanthus*, and *Helia*) of the highly advanced (Gilg, 1895) Heleae and in *Lisianthus* and *Macrocarpaea* of the Tachiinae. Comparable but somewhat simpler types of bundles are represented by the fused-ventrals in four of the twenty-five species of *Gentiana* which were studied, this genus belonging in the tribe next below the Tachiinae in advancement.

Menyanthaceae. — The floral anatomy of this group is characterized by extreme fusion of traces of different whorls, which is reflected externally in partial epigyny, and by the development of a peculiar horizontal vascular ring due to both cohesion and adnation. The anatomical features are fundamentally similar throughout the four genera studied. In both the calyx and corolla some or all of the adjacent laterals are fused in pairs and arise on radii alternating with the midribs. In no species of the Gentianaceae proper was this condition found in the corolla traces. All four genera have the base of the ovary glandular. Except in *Nephrophyllidium*, the ovary traces are four in number by fusion of the ventrals,



which ordinarily remain fused throughout the ovary. The dorsals may branch, but the main branch dominates the laterals and continues into the stigma.

Grelot (1898) has described certain internal features of the flower of *Menyanthes trifoliata*. Also worthy of record is the composition of its fusion-bundles with respect to floral symmetry. The flower exhibits a most diagrammatic arrangement when the traces making up the ten fusion-bundles are considered. Figure 14 represents the receptacle, with letters indicating the destiny of the fusion-bundles, while figure 15 shows the condition higher in the flower, each dotted circle enclosing those bundles which are fused together lower down. It is remarkable that although in a single flower the calyx and corolla lateral may arise either with its midrib or from the alternating radius, the two halves of the flower are identical. (In different flowers of this species, from two to seven of the ten corolla laterals may arise fused with the stamen traces; the flower figured has but two thus derived). Hence a bilaterally symmetrical vascular pattern is found commonly throughout the Menyanthaceae and is indicated for *Nephrophyllidium* in figure 19. The ovary of *Menyanthes* (fig. 16) shows in the placentae the prominent ovule bundles characteristic of the Menyanthaceae.

A flower of *Nymphaoides aquatica* shows three corolla laterals fused with stamen traces in their origin, and the arrangement of calyx, corolla, and stamen traces into fusion bundles is bilaterally symmetrical. In this species the five glandular protuberances at the base of the ovary become free from the ovary at their upper part, a condition not found in any other menyanthaceous species.

In *Nymphaoides peltatum* the laterals of both calyx and corolla normally arise on the radii alternating with their respective midribs; hence there are ten fusion bundles in the lower receptacle. Five (each made up of a calyx midrib, two corolla laterals, and the stamen trace) alternate with five others, each of which consists of two calyx laterals and a corolla midrib. Due to this regular alternation of two types of fusion bundles, the vascular plan displays radial symmetry in the calyx, corolla, and androecium. In

this species cohesion takes place between different radii, involving the calyx bundles just below the origin of the calyx itself (fig. 17). A slight degree of cohesion is evident in the corolla traces also, just above their point of origin, but each soon becomes quite distinct.

A very complex fusion arrangement is found in *Nymphaoides Humboldtianum*, forming a complete cohesion-adnation ring (fig. 18). Ten different types of direct connections occur, as follows: calyx midrib to one calyx lateral, calyx midrib to one or two corolla laterals, calyx midrib to stamen trace, calyx lateral to corolla lateral, corolla midrib to one or two calyx laterals, corolla midrib to two corolla laterals, and stamen trace to one or two corolla laterals.

Nephrophyllidium shows bilateral symmetry in the composition of the ten fusion bundles in the lower receptacle (fig. 19). Omitting the carpel supply from consideration, however, the alternation of corolla midribs with the combined calyx, corolla laterals, and stamen traces results in radial symmetry. Still lower in the receptacle further fusion of the ten bundles produces as few as seven bundles.

The flower is semi-epigynous, having the sepals and corolla tube attached about half-way up the ovary. Immediately beneath their point of attachment there is a complete cohesion-adnation ring (fig. 20). From this horizontal ring, the cut ends of which are circular in shape when the flower is sectioned longitudinally, differentiate all the calyx bundles, the corolla midribs, and the stamen traces. The ovary traces and the corolla midribs become distinct below the level of the cohesion-adnation ring.

Villarsia has in its lower receptacle eight fusion-bundles constituted according to a bilaterally symmetrical plan. After the four carpel traces (two dorsals, two fused-ventral pairs) split off, two of the eight fusion-bundles dichotomize to produce ten. This yields a radially symmetrical system with two types of fusion-bundles alternating, exactly as described above for *Nymphaoides peltatum*.

High in the inferior ovary of *Villarsia* (fig. 21) is found the most perfect cohesion-adnation ring known to the writer. This horizontal ring involves the entire

Fig. 1-21.—Fig. 1-7. *Centaurium umbellatum* Gilib. Origin of traces (*k*, calyx; *c*, corolla; *a*, androecium; *g*, gynoeceum; *d*, dorsal; *v*, ventral; *p*, placental) to the various floral parts.—Fig. 1. Lower receptacle.—Fig. 2. Calyx tube free.—Fig. 3. Corolla-stamen tube free.—Fig. 4. Carpel bundles distinct.—Fig. 5. Ovary showing placentation.—Fig. 6. Stigmas.—Fig. 7. Ground plan of the fundamental vascular pattern in the Gentianaceae proper.—Fig. 8-13. *Macrocarypa macrophylla* (H.B.K.) Gilg.—Fig. 8-9. Lower receptacle showing concentric fusion-bundles before and after departure of calyx traces.—Fig. 10. Formation of single unified stele after departure of corolla traces.—Fig. 11. Carpel traces differentiating with appearance of the loculi.—Fig. 12. Calyx and corolla-stamen tubes becoming free, but still fused on the radii of the last (highest) two stamen traces to leave the receptacle.—Fig. 13. Lower portion of ovary showing placentation.—Fig. 14-16. *Menyanthes trifoliata* var. *minor* Michx.—Fig. 14. Diagram showing composition of the ten fusion-bundles in receptacle.—Fig. 15. Calyx, and corolla-stamen tube, showing by dotted circles origin of various bundles from the ten fusion-bundles.—Fig. 16. Ovary showing placentation, and ovule bundles.—Fig. 17. *Nymphaoides peltatum* (S.P. Gmel.) Britton & Rendle. Ground plan of calyx bundles branching and fusion from origin to destiny, taken from 300 microns vertical extent.—Fig. 18. *Nymphaoides Humboldtianum* (H.B.K.) Kuntze. Anastomosis pattern in receptacle. Large dots, midribs; small dots, laterals.—Fig. 19-20. *Nephrophyllidium Crista-galli* (Menz.) Gilg.—Fig. 19. Receptacle showing composition of fusion-bundles. Base of loculus showing.—Fig. 20. The complete cohesion-adnation ring at a point halfway up the ovary, the calyx, corolla, stamens, and carpels being fused together at this level.—Fig. 21. *Villarsia ovata* Vent. Ovary showing placentation and the complete cohesion-adnation ring. The hollow and solid circles mark the points on this ring from which the fusion bundles indicated will differentiate at a higher level.

vascular supply except the four carpel traces, and from it five calyx-corolla and five calyx-corolla-stamen fusion-bundles alternately differentiate, as shown by the hollow and solid circles, respectively. Still higher the discrete vascular strands to the various floral parts exhibit essentially the same arrangement as in other menyanthaceous flowers.

DISCUSSION.—The classification of the Gentianoaceae which is followed most generally is that of Gilg (1895), who used the following characters to distinguish the five menyanthaceous genera from the rest of the family:

Gentianoideae.—Pollen solitary or united in tetrads, spherical or oblong, ovoid or somewhat bent, but never compressed on one side. Petals convolute or rarely imbricated in the bud. Leaves decussately opposite.

Menyanthoideae.—Pollen solitary, compressed on one side; in top view the grain appears triangular, with a germinative pore at each corner; in side view it is elliptical, or rarely almost spherical. Petals valvate in the bud, with the edges strongly recurved and fimbriate; rarely very weakly imbricated. Leaves usually originating from the rhizome, always solitary.

On the basis of these taxonomic considerations of a conventional nature, several prominent systematists have accorded full family status to the *Menyanthes* group. However, the lack of agreement among writers who have relied on the usual taxonomic criteria emphasizes the importance of characters drawn from internal anatomy, floral and otherwise, for the solution of this problem. If anatomical comparisons show many striking and consistent differences between the two "subfamilies", such evidence may well be decisive in establishing the validity of the Menyanthaceae as an autonomous family.

Perrot (1898) has carried on an extensive study of the non-floral anatomy of both the groups under discussion. The most striking fact he reported is the presence in the Gentianoideae, without exception, of sieve tubes outside the normal phloem region. Groups of these cells occur at the periphery of the pith in all stems and may occur scattered within the pith, included in the xylem of the stem, or in the xylem of the root. On the other hand, the Menyanthoideae shows no sieve tubes outside the normal phloem.

The same author also pointed out that storage of calcium oxalate crystals takes place in all genera of the Gentianoideae but is totally lacking in the other group. In the former the vascular tissue of the stem forms a typical siphonostele, while the Menyanthoideae shows discrete bundles isolated in the parenchyma, or sometimes united by sclerenchyma. Perrot called attention to the similarity of the menyanthaceous genera to each other and confessed astonishment at finding none with characters recalling those of the "Gentianoaceae proper." While retaining sub-family status for the *Menyanthes* group in order to follow Gilg's arrangement consistently throughout his paper, this French anatomist considered the evidence sufficient for regarding it as a separate family.

In characterizing the Menyanthaceae, Wettstein (1935) utilized the fact reported by Perrot (1897) that the vascular bundles are collateral, while the bundles of the Gentianoaceae are "bicollateral" or amphierbral.

Turning to the floral anatomy, the Menyanthaceae is seen to constitute a very natural group, not alone because of the similarity of its members but also by virtue of the uniqueness of their structure. The Gentianoideae affords no suggestion of the more or less complete cohesion-adnation ring herein reported. This type of fusion, best exemplified by *Nephrophyllidium* and *Villarsia*, is a most unusual feature of floral anatomy insofar as this subject has been investigated to date. Scarcely less unique are the concentric fusion-bundles found in many of the more advanced genera of the Gentianoideae but lacking in the Menyanthaceae. Both of these peculiar specializations of the vascular skeleton of the flower indicate that the two plant groups in question are on widely divergent phylogenetic lines.

Every menyanthaceous species studied shows fusion of adjacent corolla laterals on radii alternating with those of the corolla midribs. This character does not occur in any of the eighty species of the Gentianoideae which were investigated. Likewise, the latter group seems never to possess lignified vascular strands entering the individual ovules, having instead a rather richly protoplasmic parenchymatous tissue in the general placental region, which is supplied in turn by one or more lignified vertical bundles (the ventral, and its branches, if any). In marked contrast to this condition, all the menyanthaceous species which were studied exhibit conspicuous and heavily lignified ovule bundles passing horizontally from the vertical fused-ventrals into the ovules.

The pronounced tendency toward epigyny shown by the flower in the Menyanthaceae has been largely overlooked in systematic literature. In *Nephrophyllidium* and *Villarsia*, especially, the ovary is semi-inferior as a result of fusion of all the floral whorls for a considerable distance upward. The calyx segments and corolla-stamen tube of *Nephrophyllidium* become free from the ovary only at a point halfway up the latter. In several genera of the Gentianoideae, particularly in the sub-tribe Erythraeinae, the tube of the corolla is extremely thin and encircles the ovary so closely that in some cases the two actually are fused. Thus the peculiar type of epigyny which results is due to a drastic reduction in thickness of the corolla-stamen tube, the latter securing mechanical support by fusion with the ovary. The vascular traces of the corolla and androecium remain discrete in the superficial layer of cells. The epigyny of menyanthaceous genera is of a different sort, for there is no loss of thickness of the corolla-stamen tube, and the obvious external fusion is paralleled by no less marked fusion of vascular traces internally. This condition differs from the typical inferior ovary only in that the fusion does not reach the top of the

ovary, while in the Gentianaceae proper there is found no such close approach to true epigyny.

Menyanthes and *Nephrrophyllum* are closely related genera, while *Nymphoides* and *Villarsia* stand together on another short branch of descent. The last named of each pair is the more highly specialized, as indicated by the extent of fusion, both external and vascular. These four genera, together with the allied *Liparophyllum*, form a very coherent group. Although they show a stronger affinity with the Gentianaceae than with any other family, their anatomical peculiarities and the absence of various specializations found generally throughout the Gentianaceae strongly supports the separation of this group as a distinct family, the Menyanthaceae, established by Don in 1838.

SUMMARY

The validity of the Menyanthaceae, as distinct from the Gentianaceae, is considered from the standpoint of anatomy. The advanced genera of the Gen-

tianaceae often show fusion of vascular traces in the receptacle to form concentric bundles of a distinctive type. Vascular fusion in the Menyanthaceae takes a different form, producing a definite horizontal ring by cohesion and adnation, this structure being unique in floral anatomy. Additional floral characters of the Menyanthaceae which distinguish this group from the Gentianaceae are: (1) presence of ovule traces, (2) fusion of adjacent corolla laterals, (3) bilaterally symmetrical vascular pattern, and (4) epigynous insertion of floral parts in certain genera, accompanied by extreme vascular fusion. Several significant non-floral traits characterizing the Gentianaceae (siphonostele, amphicribal bundles, anomalously placed sieve tubes, and storage of calcium oxalate crystals) are lacking in the Menyanthaceae. It is concluded that the latter group merits full family status.

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CHYTRIDIACEOUS FUNGI WITH UNUSUAL SPORANGIAL ORNAMENTATION¹

F. K. Sparrow, Jr.

AMONG THE aquatic orders of the Phycomycetes, there are included in the Chytridiales some of the most curious of the fungi. Not only are these unusual in their choice of substrata, as, for example, the empty integuments of the larval stages of aquatic insects, pollen grains, other aquatic fungi, algae, eel worms, rotifers, etc., but also in their possession of remarkable and often bizarre structural features. For example, on their reproductive parts (sporangia and resting spores) some species develop unusual types of ornamentation which may take the form of spines, knobs, long, branched or unbranched hairs, broad rays, one or many spines arranged radially or in a helical series, irregular solid lobes, anastomosing ridges, or apical collarettes of plain or bipartite teeth.

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The latter, found on the sporangia of the so-called "dentigerate" species, is perhaps the most curious of all and is remarkable for its delicate beauty and precision of arrangement.

Relatively early in the investigations on chytridiaceous fungi, Rosen (1887) described the first of the algal-inhabiting, dentigerate species. These all possessed on the extramatrical, usually sessile sporangium, a crown of four small bipartite teeth which surrounded the apical exit pore. A further characteristic of Rosen's fungi was the presence within the algal cell directly beneath the sporangium of a swelling or apophysis which, from the figures given, arose as a result of the expansion of a portion of the already established rhizoidal system. To this small group of three species, Rosen applied the name "Dentigera" and considered it a distinct section of *Chytridium* in the older, inclusive sense of that genus. The species, all of which were found on Green Algae, may be characterized as follows:

Chytridium Zygnematis Rosen, sporangium spherical or ovate, the four teeth short, delicate, and strongly convergent. On *Zygnema*.

Chytridium dentatum Rosen, sporangium more elliptical, the four teeth more prominent and soon converging. On *Spirogyra*.

Chytridium quadricorne de Bary (from de Bary), sporangium short, cylindrical on a rounded base, with truncate apex, the four teeth prominent, long and scarcely convergent. On *Oedogonium*.

Of these species, only *C. Zygnematis* seems to have been studied in any detail. Numerous figures, showing "mehrbläsige Formen" (non-sessile, probably abnormal plants), "Frostsporangien" (detached young sporangia of non-sessile plants which, although frozen in the ice, remained viable), and the formation of the zoospores, are given.

All these species, as well as other, similar ones with apophysate inoperculate sporangia and posteriorly uniloculate zoospores, were later placed by Fischer (1892) in the genus *Rhizidium*, but were removed from this group by Schröter (1897) (who correctly interpreted *Rhizidium*) and placed in a new genus, *Phlyctochytrium*.

In the more than fifty years since their discovery, these dentigerate chytrids have evidently been seen but rarely (de Wildeman, 1896; Sparrow, 1932, 1933, 1936; Karling, 1932; Domján, 1936), and only a few new species² have been described (*Rhizophidium Brebissonii* (Dang.) Fisher, *R. digitatum* Scherff., *R. Hyalothecae* Scherff., etc.). In most cases, because of fragmentary observations, these new fungi are difficult to place generically. However, *Phlyctochytrium planicorne*, described by Atkinson (1909), is an unquestioned member of the *Dentigera*, distinct from the others in the possession of a crown of four plain, not bipartite teeth. The recently described *P. desmidiacearum* Dang., from the scanty information given of the sporangial state, seems doubtfully distinct from *P. quadricorne*; it is of interest, however, from the fact that it is unquestionably capable of attacking living desmids which respond to the incursions of the fungus (as Scherffel, 1925, has observed in other instances) by the formation of wall material around the penetration tube of the invader. Smooth-walled, spherical, extramatrical resting spores were also found in this species, constituting probably the first undoubted instance of their occurrence in the *Dentigera* group.

In a collection of Green Algae from Crooked Lake, Washtenaw County, Michigan, made October 15, 1937, there developed a number of chytrids among which were found five different dentigerate species of *Phlyctochytrium*, three of which were new. As these fungi (fig. 1-3) were not essentially different in method of development from Rosen's *P. Zygnematis*, their chief interest lies in the variety of their ornamentation. They all occurred on both bright green

and obviously dead algal filaments, and all were able to maintain themselves in varying degrees on boiled plants of *Cladophora* and *Oedogonium*.

PHLYCTOCHYTRIUM PLANICORNE Atkinson. — This fungus, described in 1909 by Atkinson on *Spirogyra varians*, has as its distinctive feature an apical crown of four plain teeth surrounding the exit pore of the sporangium. The extramatrical body was described as broadly elliptical, $6 \times 8 \mu$, and the intramatrical part as consisting of a globose apophysis 3μ in diameter, from which several branched, feebly developed rhizoids radiated. The zoospores were not seen. In 1932 (Sparrow) I described material of this species found on *Rhizoclonium heiroglyphicum* at both Cold Spring Harbor, Long Island, New York, and Cambridge, Massachusetts, which had narrowly to broadly ovate sporangia, differing little from Atkinson's fungus in size but usually being longer than wide. The teeth were plain and four in number, and the intramatrical apophysis varied from a slender fusiform enlargement to a definitely spherical structure. The rhizoidal system as well showed considerable differences in degree of development, consisting in some cases of a few tenuous, branched threads emerging from the surface of the apophysis, while in others it was extensive and relatively stout, particularly where it joined the sub-sporangial swelling. The zoospores emerged partially differentiated, apparently surrounded by a vesicle, and after completing their development outside the sporangium, burst the vesicle and swam away.

The present material was found in greatest abundance on dead, colorless filaments of *Cladophora* and to a lesser degree on *Oedogonium*, in company with *Catenaria* sp., *Rhizophidium chaetiferum* Sparrow, *Diplophlyctis laevis* Sparrow, and the other dentigerate species shortly to be described.

The establishment of the thallus and its subsequent development were not observed in great detail but were essentially as follows: The body of the quiescent zoospore on the outer surface of the alga produces a slender tube which penetrates the wall for a varying distance before branching (fig. 1). Coincident with this, the extramatrical part becomes somewhat narrowly ellipsoidal, and on it there are soon visible four apical protuberances (fig. 1). Further development of the rhizoidal system involves the elongation, branching and rebranching of the delicate filaments, and the expansion of that portion of the rhizoid immediately beneath the face of the inner wall of the alga (fig. 1, 2). This expansion eventually becomes the apophysis, which, while variable in size and shape, is always present in this species.

As the extramatrical portion—the future sporangium—matures, it steadily enlarges and becomes narrowly pyriform, the broader portion resting on the wall of the alga (fig. 3). The apical collar of four (very rarely 6; fig. 6) highly refractive, slightly converging teeth becomes increasingly prominent, and when fully formed strongly resembles the set of prongs used in certain familiar types of gem settings. Occasionally, non-sessile, stalked sporangia were found.

² Graff (1928) has shown that the two "teeth" of *P. equale* Atkinson are in reality the walls of the short discharge tube viewed in optical section.

These were produced when zoospores germinating at some distance from the algal wall eventually made contact with it and formed a vegetative system within. Hence, the sporangium when it developed was not resting directly on the algal wall, but was separated from it by a short length of germ tube which remained isodiametric or became somewhat swollen. It seems evident then that the formation of such "mehrbiasigen Formen," as they were termed by Rosen (1887), is dependent upon the relative positions of the germinating spore and the substratum, and is therefore of no taxonomic significance.

The rhizoids are eventually drained of their contents, with the exception of a large oil globule which generally remains in the apophysis (fig. 5). The receptive, extramatrical part becomes transformed into a sporangium. Whether or not a wall is laid down, as is likely, separating the sporogenous and vegetative elements could not be determined. The irregularly disposed, small droplets of oil found in the immature sporangial fundament (fig. 2) flow together to form more regularly spaced, larger refractive globules. When conditions are favorable for spore discharge, the apical part of the sporangium wall within the collarette of teeth deliquesces, the prongs are tilted back slightly, and some of the zoospores emerge singly without ciliary motion (fig. 4). Outside they remain motionless or wave slowly back and forth, still attached to the sporangium each by its single posterior cilium. Those remaining within may creep about amoeboidly before finally emerging. Eventually, most of the discharged spores initiate ciliary movement, preceded by a violent trembling of the body, and then dart away. A few may crawl about in amoeboid fashion for a time before finally leaving the vicinity of the sporangium. As may be seen, this account of spore discharge differs from that previously described for this species. Such variations are common among these fungi, and the presence or absence of a vesicle (if, indeed a discrete structure is ever formed) is probably dependent upon environmental conditions prevailing at the time. A similar situation has been described by Scherffel (1925) in *Rhizophidium parasitans* Scherffel, and I have observed it in many other chytrids.

The sporangium at maturity was 9–17 μ in diameter by 15–24 μ in height, including the solid refractive teeth, which were seldom more than 4 μ in length. The apophysis when spherical was 4–13 μ in diameter, when sub-spherical, up to 12 μ wide by 10 μ high. The rhizoids, which emerged from one or more places on the apophysis, were variable in length, seldom exceeding 50 μ . The zoospores contained in the clear plasma of their spherical body, which was 4–6 μ in diameter, a few minute droplets and a prominent spherical, slightly eccentric highly refractive oil globule about 3 μ in diameter. The single trailing cilium was about 30 μ in length.

PHLYCTOCHYTRIUM BULLATUM SPARROW. — In a paper on inoperculate chytrids collected in the vicinity of Ithaca, New York (Sparrow, 1933) I recorded

some fragmentary observations on a fungus found on *Cladophora* which, because of the formation of remarkable flange-like, solid outgrowths on the sporangium, I believed to be a new species probably belonging to the genus *Phlyctochytrium*. No discharge of zoospores was seen, and the organism was figured but left unnamed with the hope that future observations might settle the question of its generic affinities.

The Michigan collection of *Cladophora* yielded this same fungus in fair abundance. It occurred primarily on the bright green and, to a lesser degree, on colorless algal filaments. Very rarely it was found on dead *Oedogonium*. The sequence of development is like that previously described for *P. planicorne*, and, as in that species, the outgrowths appear very early on the developing sporangial fundament.

The extramatrical sporangium is sub-spherical or broadly urn-shaped and rests on the outer surface of the algal wall (fig. 13). In rare instances stalked, non-sessile forms are found. The wall of the sporangium is colorless and bears six regularly placed, radially arranged, broad, flange-like, solid, highly refractive bosses, the apices of which are strongly incurved and convergent. At the tips of each of these remarkable and striking outgrowths is found a deeply incised, divergently bipartite tooth (fig. 7, 8). Immediately surrounding that part of the sporangial apex which will deliquesce to form the discharge pore, and within the outer whorl of bosses, there is found a second collarette composed of four minute, bipartite, sessile teeth (fig. 9, 11). While this second, inner row of minute teeth was not mentioned in the description of the Ithaca material (Sparrow, 1933), it is evident from an examination of figures 1 and 16 that it was unquestionably present.

Great difficulty was experienced in obtaining adequate information on the nature of the rhizoidal system in this species, largely due to the density of the green content of the *Cladophora* cells. In general, when growing in *Cladophora* and *Oedogonium*, the spherical or broadly fusiform subsporangial apophysis gives rise at one side to a single broad filament which eventually branches and which may ramify through more than one algal cell (fig. 10, 13). The rhizoid emerging from the opposite side of the apophysis is usually strongly suppressed in its development and, if present at all, seldom consists of more than a few delicate, branched, tapering threads (fig. 12, 14). Very rarely a more radially symmetrical vegetative system is formed (fig. 7).

The zoospores emerged from the sporangium upon the deliquescence of a circular area of the apical wall immediately within the second whorl of four minute teeth. They escaped individually; without ciliary or amoeboid motion, and rested in a loose group before the exit pore, the ends of their cilia still retained within the sporangium. After a few minutes a faint jerking of the body was initiated by one of the spores, which increased in intensity and which seemed almost instantly to be transmitted to the whole group.

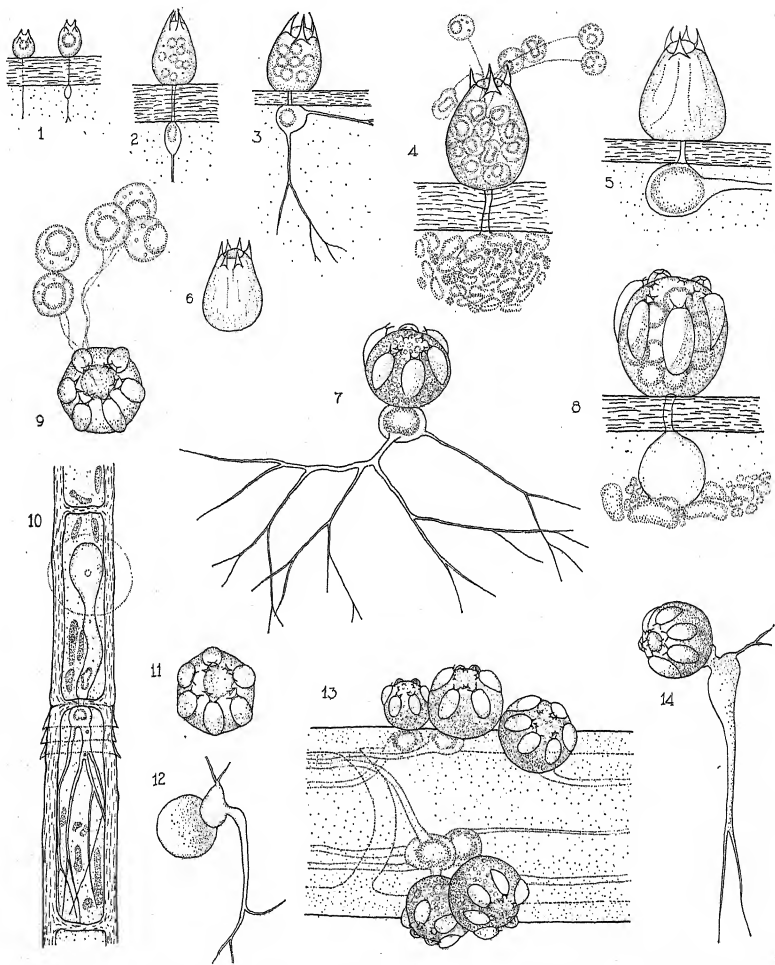


Fig. 1-14.—Fig. 1-6 (all $\times 1000$). *Phlyctocytrium planicorne* Atk. on *Cladophora* sp.—Fig. 1. Early stages in the development of the thallus.—Fig. 2. Immature sporangium and a portion of the rhizoidal system.—Fig. 3. Mature sporangium with globules of zoospores organized.—Fig. 4. Discharge of zoospores.—Fig. 5. Empty sporangium showing exit pore surrounded by teeth, and spherical apophysis containing a large residual oil globule.—Fig. 6. Sporangium with six plain teeth.—Fig. 7-14. *Phlyctocytrium bullatum* Sparrow.—Fig. 8, 9, 11-14 on *Cladophora* sp., Fig. 7, 10 on *Oedogonium* sp.—Fig. 10, 12-14, $\times 600$; fig. 7-9, 11, $\times 1000$.—Fig. 7. Mature plant with algal wall omitted to show intramatrix part.—Fig. 8. Unusually large sporangium with spherical apophysis.—Fig. 9. Sporangium in top view showing a motionless cluster of recently emerged zoospores.—Fig. 10. Rhizoidal system in

One by one, the spores tugged loose from the sporangium and darted away. The bodies of the swimmers were spherical and composed of clear cytoplasm of low refractivity, within which was a large, highly refractive, usually eccentric oil globule and a few minute droplets (fig. 9). The single posterior cilium was 6-7 times the diameter of the spore body in length. No resting spores were found.

This fungus differs from other dentigerate types in the possession of the prominent bosses (resembling in this respect *Cladocytrium cornutum* de W. (Wildeman, 1896), each of which is terminated by a single deeply incised tooth.

Phlyctocytrium bullatum Sparrow.³ — Sporangium sub-spherical or broadly urn-shaped, 10.5-23 μ high by 12-26 μ in diameter, colorless, with two concentric whorls of solid, apical, converging teeth: the innermost circle of four minute, divergently bipartite sessile ones which immediately surround the discharge papilla; the outermost of six bipartite, strongly diverging, longer ones each of which terminates the inwardly arching tip of a broad flange-like solid boss 5-7 μ long by 3 μ wide by 3-5 μ high. Intramatrix system composed of a broadly fusiform, sometimes spherical or irregular sub-sporangial swelling, 10-20 μ wide x 6-10 μ high, from one side or occasionally opposite sides of which emerges a wide rhizoid which usually branches at some distance from the swelling; the rhizoids ramifying through one or more cells of the alga. Zoospores spherical, 8 μ in diameter, the clear plasma containing a single large, slightly eccentric spherical or hemispherical oil globule, 4-5 μ in diameter, and a few minute, peripheral granules; possessing a single posterior cilium about 40 μ in length. Resting spores not observed. Saprophytic and weakly parasitic on *Cladophora* sp.

PHLYCTOCYTRIUM DENTIFERUM Sparrow. — The third of the dentigerate species occurred in greatest abundance on obviously dead *Cladophora*, although it was occasionally found on bright green filaments. Also, it could be cultivated on boiled *Cladophora* and *Oedogonium*.

Development of the fungus and maturation of the sporangium presented no unusual points of interest, being similar in these respects to *P. planicorne* previously described. The sporangium was sub-spherical or ovate and rested with its usually somewhat flattened base on the outer surface of the algal wall (fig. 18). Occasionally, as in other species, non-sessile, stalked specimens were found (fig. 17). In the apical region of the extramatrix part there was differentiated early in the development a double collarette of bipartite teeth (fig. 15). The outermost whorl consisted of six radially arranged, sessile, or slightly elevated, solid, highly refractive teeth; the inner was made up of four smaller, sessile ones which imme-

diately surrounded the rather prominent apical exit papilla. Within the alga and connected to the extramatrix part by a tube of varying width, there was formed the nutrient-gathering system. This consisted of a branching series of rhizoids of varying length and complexity and, as a secondary development, an apophysis. This was broadly or narrowly fusiform, spherical or irregular, and was generally formed immediately beneath the inner face of the algal wall (fig. 16, 18, 19). Occasionally, however, complete penetration of the wall did not take place, and the rhizoidal system developed between the layers of wall material (fig. 17). In these cases the apophysis was always strongly flattened. In contrast to *P. bullatum*, the rhizoids of *P. dentiferum* were never observed to ramify through more than one algal cell.

Discharge of the zoospores could be induced in temporarily resting sporangia by transferring infected algal filaments to fresh distilled water. The first indication of discharge was the slight change of shape assumed by the prominent oil globules of the enclosed spores. This was probably caused by movement of the plasma of the spore bodies themselves, although such motility could not actually be detected. A gradual but steady elongation and expansion of the apical papilla then ensued (fig. 20), accompanied by a dilation of the whole apical region. As a result of this expansion the teeth were thrust farther apart and, as the first spore emerged (fig. 21), were even tilted outwardly. During the slow emergence of the spores, the large globule constantly changed shape (fig. 21-24). Once the major part of the body of the first spore had emerged there was a noticeable increase in the size of the oil globules still within the sporangium. These changed their shape and position constantly as the spores made their way to the exit pore (fig. 25-28). At what moment the deliquescence of the delicate apical wall of the papilla occurred could not be detected even at high magnifications. Outside, the escaped swimmers remained motionless or slightly swaying, the ends of their intertwined cilia still retained within the sporangium (fig. 29). The spherical body of the spore, composed of clear plasma within which were embedded a few minute granules, possessed a very large spherical, eccentric oil globule. It was remarkable to note how greatly the escaped spores and their globules increased in size after emerging from the sporangium (fig. 20, 29). After a short period, never more than five minutes, individual movement was initiated, the cilia were jerked free from the sporangium, and the spores after a few preliminary hops darted off. Following discharge, the apex of the now empty sporangium contracted to its original size (fig. 30). On this the arrangement of the teeth could be clearly seen and the two concentric whorls of orna-

³ Latin, but not English, descriptions of *P. bullatum*, *P. dentiferum*, and *P. ureolare* have been published in Bull. Boston Soc. Nat. Hist. 8: 295-296. 1937.

Oedogonium, showing polyphagous character.—Fig. 11. Top view of empty sporangium showing the 6-4 arrangement of the 10 teeth.—Fig. 12, 14. Plants drawn with algal wall omitted to show nature of rhizoidal system.—Fig. 13. Group of sporangia on the surface of a filament of *Cladophora*, the content of the latter omitted to show rhizoidal systems.

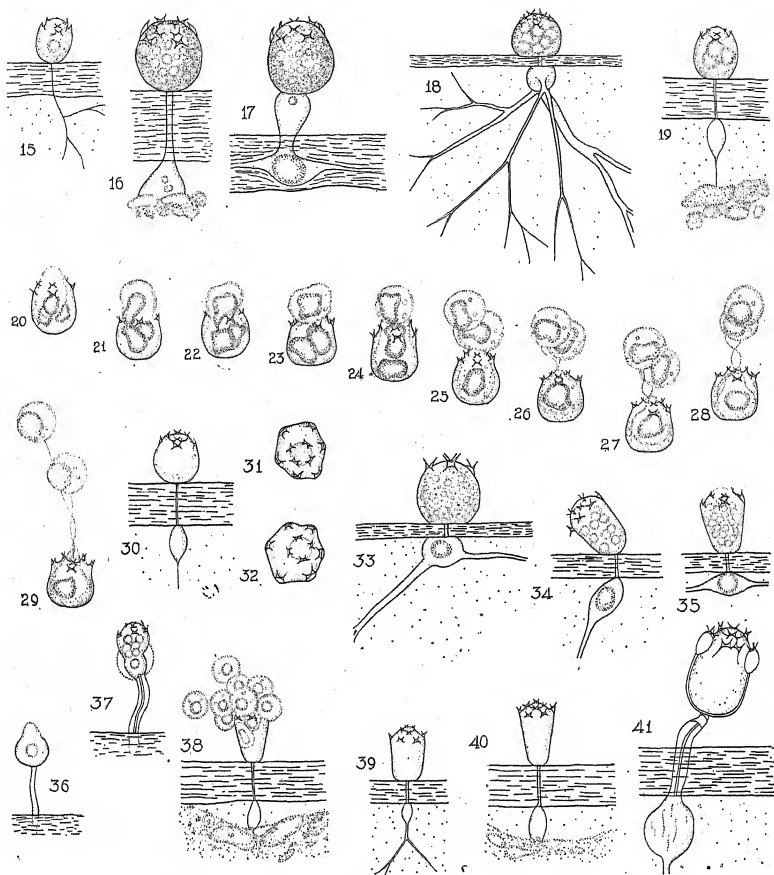


Fig. 15-41.—Fig. 15-32. *Phlyctocytrium dentiferum* Sparrow. All, save figure 18, on *Cladophora* sp. and $\times 1000$; fig. 18, on *Oedogonium* sp. and $\times 600$.—Fig. 15. Early stage in the formation of the thallus; rhizoids established, teeth visible.—Fig. 16. Nearly mature sporangium with broad penetration tube, the two whorls of teeth completely formed.—Fig. 17. Nearly mature sporangium with the rhizoids and apophysis developed between the layers of the algal wall.—Fig. 18. Mature sporangium on *Oedogonium*.—Fig. 19. Small, mature sporangium containing three zoospores.—Fig. 20-29. Stages in the discharge of the zoospores. Note expansion of apex and the change in shape and size of the oil globules of the spores.—Fig. 30. Empty sporangium.—Fig. 31, 32. Top views of empty sporangia showing double collarette of ten teeth arranged in 6-4 fashion.—Fig. 33. Sporangium of undetermined species (*P. quadricorne* de Bary ?) with collarette of four teeth.—Fig. 31-41. *Phlyctocytrium urceolare* Sparrow. All on *Cladophora* sp. and $\times 1000$.—Fig. 34, 35. Mature sporangia showing variations in shape of apophysis and position of sporangium.—Fig. 36. Very young stage in the development of a non-sessile plant showing the pyriform shape assumed by the growing spore body. The intramatrix part is not shown.—Fig. 37. Constricted, stalked sporangium partially surrounded by a cup-like shell (wall of a previous sporangium ?).—Fig. 38. Discharge of the zoospores.—Fig. 39, 40. Empty sporangia showing typical shape.—Fig. 41. Non-typical, giant sporangium with teeth of outer whorl doubled.

ments easily observed, particularly in apical view (fig. 31, 32).

Since in the general shape of its sporangium, *Phlyctochytrium dentiferum* resembles *P. quadricorne* and, to a lesser degree, *P. zygnematis*, it might easily be confused with these species unless the apical region is carefully examined. Indeed, the question might well be raised whether or not the first described *Dentigera* did not actually possess two whorls of teeth. While such might be possible, dentigerate forms with only four teeth have been observed in several instances since Rosen's (1887) time (Karling, 1932, fig. 19; Sparrow, 1933, 1936). In any case there is little reason to doubt the observations of de Bary and Rosen on this point, particularly since the latter has figured apical views of several sporangia in all but one of which (Rosen, l.c. below fig. 23) only four teeth are visible. The exception, however, shows definite evidence of possessing more than four but less than ten teeth, the number uniformly found in the present material. Furthermore, there occasionally occurred in the Michigan collections a fungus with a nearly spherical sporangium on which was borne an apical crown of four strongly bipartite teeth (fig. 33).

Phlyctochytrium dentiferum Sparrow. — Sporangium slightly sub-spherical, 10–15 μ high by 10–14 μ in diameter, colorless, with two apical concentric whorls of solid, converging teeth: the innermost circle of four minute (about 2 μ high by 2 μ wide), divergingly bipartite, sessile ones which immediately surround the discharge papilla; the outermost of six larger (about 4 μ high by 2.5 μ wide) bipartite sessile or slightly elevated ones. Intramatrical system composed of a broadly fusiform, spherical or irregular sub-sporangial swelling, 5–15 μ in diameter by 5–12 μ in height, from opposite sides of which emerges a moderately broad, eventually branching rhizoid. Zoospore spherical, 7 μ in diameter, with a single large, slightly eccentric oil globule 4 μ in diameter; possessing a single posterior cilium about 30 μ in length. Resting spores not observed. Saprophytic and weakly parasitic on *Cladophora* sp.

PHLYCTOCHYTRIUM URCEOLARE SPARROW. — The fourth of the dentigerate species found in the present collection usually occurred in dense clusters on bright green, as well as dead filaments of *Cladophora*. It was also grown on boiled filaments of *Cladophora* and *Oedogonium*, but did not seem to thrive on these substrata.

The development of the fungus offered no points of interest except that the body of the spore in the early stages of growth usually became strongly pyriform by the formation of a prominent apical papilla (fig. 36). As in the other, previously described species, non-sessile plants were frequently found (fig. 36, 37), as well as many others exhibiting variations in the shape, size, etc., of the subsporangial apophysis (fig. 34, 35, 40).

The outstanding characteristic of *P. urceolare* is the shape of its sporangium. This, while more variable in form than in the preceding species, is predominantly cylindrical with a rounded base and is slightly expanded distally up to the place of emergence of the

outer whorl of teeth. At this point it tapers sharply toward the blunt, rounded apex which is surrounded by a second, inner whorl (fig. 34, 35, 39, 40). As in *P. dentiferum* and *P. bullatum* there are ten bipartite solid teeth in two whorls arranged radially in 6–4 fashion. The outer collarette of six, probably sessile, is upright or even tilted outwardly, whereas the inner group, composed of four smaller, sessile ones, on undischarged sporangia converges slightly toward the apical papilla (fig. 35). After discharge both sets are generally upright (fig. 39, 40). Occasionally, sporangia are found which are strongly constricted in the mid-region. A close examination of these nearly always reveals the presence of a delicate, cup-like, loose-fitting membrane surrounding the lower half of the body. In non-sessile examples, this is always accompanied by a stalk with a very narrow lumen (fig. 37). While stages in the development of these sporangia were not seen, it is probable that they had developed within older ones, possibly from undischarged spores. In one case (fig. 41) an empty, unquestionably abnormal sporangium was observed. It was non-sessile and at least twice the size of the other plants, with its outer whorl of ornaments consisting of double, bipartite teeth borne at the tips of small solid bosses. This aberrant plant was of particular interest because of the presence of the bosses. Such a condition suggested that on the smaller, normal sporangia these teeth were not sessile, but borne on elevations so small that they escaped detection.

The posteriorly uniciliate zoospores, 8–16 of which were formed in a sporangium, emerged in exactly the same fashion as in *P. dentiferum* (fig. 38). They possessed a spherical body with a large, central or slightly eccentric oil globule and a few minute droplets in the clear plasma. The spores, after remaining in a motionless group at the mouth of the sporangium for a few minutes, initiated individual movement and suddenly darted away.

This fungus is probably identical with that incompletely observed on *Cladophora* from Ithaca, New York, (Sparrow, 1933, fig. 1, 3), which was tentatively assigned to *Phlyctochytrium*. When the present material was first observed it was thought that the cylindrical shape of the sporangium resulted from the dense crowding of the plants on the algal wall and that it was in reality only a form of *P. dentiferum*. However, further investigation revealed many isolated sporangia all of which maintained the typical cylindrical, slightly funnel-shaped aspect. For this reason it seems evident that we are in reality dealing with a distinct species of *Phlyctochytrium*.

Phlyctochytrium urceolare Sparrow. — Sporangium colorless, somewhat variable in shape but predominantly cylindrical and expanding slightly distally until reaching the first whorl of 6 sessile, bipartite, solid, upright or slightly diverging teeth, where it tapers sharply toward the apical discharge papilla which is surrounded by a whorl of 4 minute bipartite, upright, solid teeth; 10–14 μ high by 7–11 μ in diameter, tapering to 5–6 μ at the apex. Intramatrical system composed of a narrowly to broadly fusiform or occasionally spherical sub-sporangial swelling,

2-5 μ in diameter by 7-10 μ in height (3-7 μ in spherical examples); the swelling if narrowly fusiform generally bearing at its base a single rhizoid which ultimately branches; if broadly fusiform or spherical, bearing two oppositely-placed rhizoids which eventually branch. Zoospore spherical, 4 μ in diameter, with a single slightly eccentric, spherical oil globule 2 μ in diameter and a single posterior cilium about 20 μ in length. Resting spores not observed. Saprophytic and weakly parasitic on *Cladophora* sp.

DISCUSSION.—The foregoing account of these dentigerate species of *Phlyctochytrium* involves certain points of more general interest which merit further consideration. Perhaps the most significant of these is that in a single collection of algae three of the four representatives of a particular chytridiaceous genus were new species. Furthermore, the present paper does not deal with all the new or unusual chytrids which were obtained from this material. These, which will be described in other papers, include new species of *Rhizophidium*, *Diplophycis*, possibly *Chytridium*, and, what was perhaps even more interesting, a form seemingly belonging to a monotypic genus founded over sixty years ago and apparently unobserved since that time. This abundance of new species emphasizes what I have stressed on more than one occasion—namely, that we have by no means attained a knowledge of the aquatic fungi comparable with that of the flowering plants or, in fact, with that of most groups of the higher fungi.

A second point of interest is the remarkable constancy, regularity, conventionalization of structure, and symmetry of arrangement exhibited by the sporangial outgrowths. In *P. planicorne* these so-called "teeth" were never cleft as in the other species, but were always solid, fairly slender spikes with concave bases. In *P. bullatum*, *P. dentiferum*, and *P. urceolare*, on the other hand, they were uniformly apically cleft in varying degrees. In *P. bullatum* the teeth of the outer whorl of six were especially deeply cleft, so much so that each tooth usually appeared as two slender, needle-like, strongly diverging structures. Those of the inner whorl of *P. bullatum*, as well as those of both whorls of the remaining species, were less prolonged (i.e., shallowly cleft) and were sessile or nearly so on the wall of the sporangium. In all three cases they were solid and refractive, with strongly diverging apices. In some instances the teeth composing the outer collarette of *P. dentiferum* and *P. urceolare* appeared to be borne at the tips of very small bosses, although this could not be verified with certainty. If such were true, it would serve to explain the appearance on the giant form of *P. urceolare* (fig. 41) of definite bosses which, because of enlargement, are more easily visible.

Great uniformity was also observed in the number and arrangement of the teeth on the sporangium. Among the hundred or more sporangia of *P. planicorne*, only one (fig. 6) was observed to have more than four. The remaining species invariably possessed 10 teeth in the characteristic 6-4 arrangement, except

for the single giant form previously mentioned. While the plant shown in figure 33 might be considered a variation of *P. dentiferum*, it seems more likely to be allied to de Bary's *P. quadricorne* or to *P. Zygnematis* Rosen.

While attention has been directed in this paper primarily to a morphological consideration of these chytrids, something might be said of their parasitism and host relationships. Rosen (1887) pointed out that, while he termed *P. Zygnematis* a parasite, it was perhaps more of a saprophyte and attacked only such cells as were already in poor health because of unfavorable environmental conditions. He was led to this conclusion by two facts—first, that newly liberated zoospores only rarely attacked healthy cells, whereas the surfaces of obviously dead ones were crowded with germlings, and secondly, that green cells were attacked only in cultures of *Zygnema* in which, because of restricted environmental conditions, the filaments were showing signs of poor health. A similar situation was noted in the Michigan material. *Phlyctochytrium planicorne*, however, was an exception, always occurring on dead, whitened filaments of the algae. Since the species herein described were all capable of maintaining themselves on boiled filaments of *Cladophora* and *Oedogonium*, it can be definitely said that they may exist as saprophytes. However, growth on such killed cells proves only that the fungus is capable of leading a saprophytic existence. It does not eliminate the possibility that, at certain times, it may be weakly or virulently parasitic. As evidence that species closely allied to those discussed in this paper may attack living algal cells, we have the recent findings of Dangeard (1937). This investigator observed that cells of *Closterium* attacked by the zoospores of *Phlyctochytrium desmidacearum* Dang. are stimulated to produce wall material which may completely enclose the germ tube of the invading fungus. As this germ tube elongates and penetrates farther and farther into the lumen of the host cell, the wall substance continues to be formed, and eventually the parasite is exhausted of its own material and dies. By this means the host saves itself from the ravages of the fungus. Similar, though less successful instances have been reported by Scherffel (1925) in other algae, and I have repeatedly observed it, particularly in attacked cells of *Tribonema bombycina*. It does not seem probable in these cases if the algae were dead or even moribund that they would still possess the power to form these protective plugs of new cellulose. We may conclude, therefore, in such instances that the cytoplasm is still viable.

SUMMARY

Among the aquatic chytridiaceous fungi attacking algae are found species which possess remarkably symmetrical and conventionalized outgrowths on their reproductive organs. The present paper deals with certain of these species belonging to the genus *Phlyctochytrium* which were found in a single collection of Green Algae in a lake near Ann Arbor, Michigan.

All these forms develop on their sporangia one or two apical collarettes of solid, refractive teeth. In *P. planicorne* Atk., found on dead filaments of *Cladophora* and *Oedogonium*, the extramatrix, pyriform sporangium possesses a crown of four solid, uncleft teeth, which surrounds the circular exit pore through which the zoospores escape. Within the alga and connected to the sporangium by a slender tube which passes through the thick wall of the substratum is a branched system of delicate, tapering rhizoids. These seem to emerge from the surface of a distended, sub-sporangial apophysate structure which, however, actually makes its appearance after the establishment of the rhizoids. The zoospores are fully formed within the sporangium and escape upon the deliquescence of a circular area of the sporangium wall, immediately within the crown of teeth. Each of the spores possesses a spherical body in which is a prominent, highly refractive oil globule and a single, long posterior cilium. After a period of swarming the spore settles down on the outer surface of the algal wall, retracts its cilium, and penetrates the substratum, the body eventually forming the new sporangium. No resting spores were found in this or any of the species studied.

The remaining fungi possess the same general structure, development, and reproductive cycle but differ from *P. planicorne* and from each other in the ornamentation and shape of their sporangia. All bear at the apex of the sporangium 10 teeth arranged

in 6-4 fashion in two concentric whorls. Each tooth is deeply incised (bipartite) rather than plain. On the sub-spherical or broadly urn-shaped sporangium of *P. bullatum* each of the six teeth of the outer whorl is formed at the incurved tip of a highly refractive, large, flange-like, solid boss, an outgrowth of the sporangial wall. Within this whorl and immediately surrounding the area which deliquesces to form the exit pore is a second collarette composed of four minute, sessile, solid, bipartite teeth. On the sub-spherical sporangium of *P. dentiferum* the members of both whorls are sessile or nearly so, the outer six being somewhat larger than the inner four. *Phlyctochytrium urceolare* differs from *P. dentiferum* in its possession of a more cylindrical sporangium which is slightly expanded distally up to the place of emergence of the outer whorl of six sessile teeth. There it tapers sharply towards the blunt, rounded apex which is surrounded by the inner whorl.

All the fungi studied may live saprophytically on dead cells of *Cladophora* and, to a lesser degree, on *Oedogonium*. All save *P. planicorne* have also been found on bright green filaments of *Cladophora*, which suggests that they may at times be weakly parasitic. Diagnoses in English are given of the three recently described species, *Phlyctochytrium bullatum*, *P. dentiferum*, and *P. urceolare*.

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THE LIFE HISTORY OF EISENIA ARBOREA¹

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THE PURPOSE of this paper is to offer additional evidence in support of the view that antithetic alternation of generations is generally present throughout the order Laminariales. It reports a study of the life history of *Eisenia arborea* Aresch. in which special attention was given to the development and behavior of the gametophytes.

The earliest study of reproduction in the order Laminariales dealt only with the sporophyte bodies, while recent studies have greatly extended the knowledge of the complete life histories of this group. The reproductive organs were first studied by Thuret (1850), who considered the product of the unilocular sporangia to be zoospores. Williams (1900) considered these motile spores to be asexual and described the "branched protonema-like structure" which developed from the zoospores. The studies of Drew (1910), Killian (1911), and Williams (1912), suggested further investigations and led to Sauvageau's (1915, 1916) studies in which a definite alternation of generations was demonstrated. Kylin (1916, 1918) published his work and confirmed the results of Sauvageau in all essential particulars. Ikari (1921) included a description of the gametophytic generation of *Laminaria religiosa* which agreed with the results of previous studies. Since then the following investigations have added to our knowledge of the process of alternation of generations in the order: Williams (1921) made a preliminary study on *Laminaria* and *Chorda*; Brandt (1923) reported on the life history of *Macrocystis pyrifera*; Myers (1925, 1928) described the alternation of generations in *Postelsia palmaeformis*, *Laminaria sinclairii*, and *Egria menziesii*; Delf and Levyn (1926) on *Macrocystis pyrifera*; Angst (1927, 1929) on *Costaria costata* and *Pleurophyucus gardneri*; Hartge (1928) on *Nereocystis luetkeana*; and McKay (1933) on *Pterygophora californica*.

It appears that among the giant kelp the conspicuous generation is the sporophyte, while the gametophyte is microscopic in size. If there are any exceptions, they are yet to be discovered.

The genus *Eisenia* was established by J. E. Areschoug in 1876 when he received a plant collected by Dr. Gustav Eisen at Catalina Island, off the southwest coast of California. It was again described by Areschoug (1884) and assigned a place near the genus *Postelsia*. The development and systematic position were first made clear by Setchell (1905).

The distribution of *Eisenia arborea* is limited to the coast of California, south of Point Conception to some unknown point south of San Diego, California. It grows in the sublittoral zone on rocky shores where the surf is strong and the breakers dash over it at low tide. The mature sporophyte stands erect and

is usually one-half to three-fourths of a meter in height.

COLLECTION AND METHOD OF CULTURE.—Monthly collections were made at Laguna Beach, California, over a period of a year. Sporophylls were placed in glass jars of sea water or wrapped in moist paper, placed in a wet sack, and brought to the laboratory. Pieces of the sporophyll were washed thoroughly in sterilized sea water, using a stiff brush to remove, as nearly as possible, all foreign matter which adhered to the gelatinous surfaces, as difficulty was always experienced in setting up cultures that were free from contamination. The culture solution used in the present investigation was essentially the same as that recommended by Kylin (1916). To further minimize contamination, all glassware, slides, and sea water were sterilized in the autoclave at 15 pounds for 30 minutes. Sodium nitrate in the ratio of one gram per liter and a small crystal of potassium phosphate were added to sea water. In order to increase the amount of oxygen after sterilization, the water was poured back and forth several times from a height of 20–30 cm. It was found best to sterilize the sea water just before using, since a precipitation of crystals formed when the water was allowed to stand.

A specially constructed glass-enclosed culture cabinet was used and placed on the north side of the laboratory building where it was never exposed to direct sunlight. A thermostat in the culture cabinet kept the temperature within two degrees of that desired. Glass tumblers of 250 cc. capacity were used so that in case of contamination one or several might be discarded without destroying all cultures from any particular collection. Glass slides were placed around the sides which allowed for easy removal for observation from time to time. Ten to twelve fragments of sori about one centimeter square were placed in each dish and allowed to stand from 12 to 24 hours before being removed. It was found that temperatures ranging from 8–12°C. were best for gametophyte development and growth.

THE SPOROPHYTE.—The smallest plants observed are about 6 cm. high and are not easily distinguished from young plants of *Egria*. The holdfast consists of simple hapters, the stipe is short, and the margins of the blade entire. In plants slightly larger the important generic differences are on the margins of the blade, where, especially toward the base, there are several coarse toothlike outgrowths (fig. 1b). These outgrowths increase in size and number, until they give the blade a pinnate appearance. Modifications appear which finally result in the characteristic frond of *Eisenia* (fig. 1a). The basal margins of the blade begin to thicken and to turn in on one side. While this is going on, the blade becomes shorter and wider, the fronds longer and more toothed.

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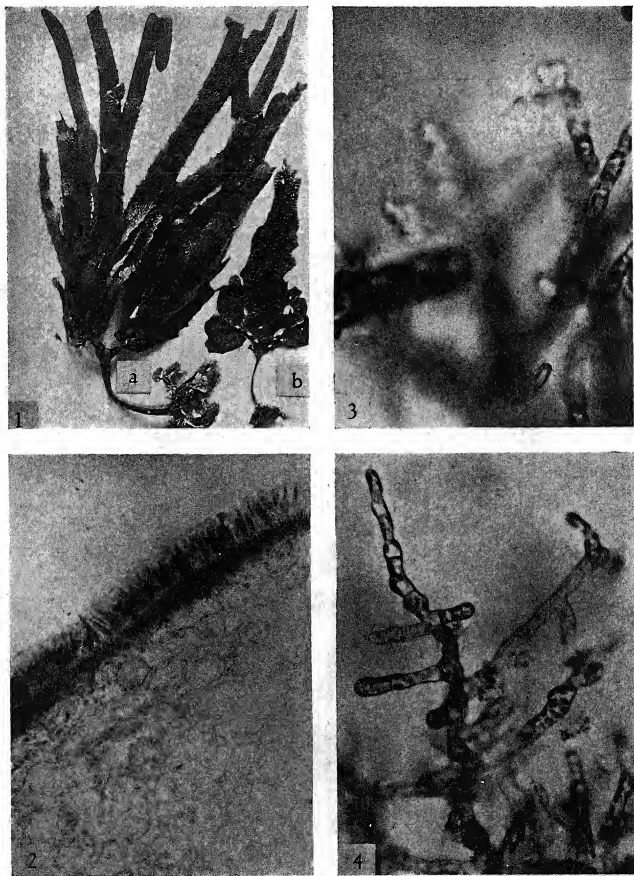


Fig. 1-4. *Eisenia arborea* Aresch.—Fig. 3-4. Plants grown upon slides. All of plant can not be seen in one focal-plane. $\times 1000$.—Fig. 1. a, Mature sporophyte with holdfast, stipe and fronds; b, Immature sporophyte.—Fig. 2. Cross-section of sporophyll showing sporangia with spores.—Fig. 3. Microgametophyte with empty antheridia.—Fig. 4. Microgametophyte.

After a time the blade wears away gradually until it disappears except for the small side pieces which lead to the formation of two arms supporting the fronds or sporophylls. The arms become thickened, increase in length to 30 cm. or more, and make a half twist. The sori are small and oblong or irregularly elliptical in shape, a few centimeters long and proportionally narrow. As they continue to produce sporangia, they

become confluent into fairly large patches of irregular outline nearly covering the entire surface of the older sporophylls. A condition not described by Setchell exists in that the sori begin to develop at the base of the sporophylls and rarely are seen to extend to the apex.

DEVELOPMENT OF THE SPORANGIA.—The sporangia (fig. 2) of *Eisenia arborea* are borne in irregular

shaped, dark colored sori which occur on both surfaces of the sporophylls. The sorus is made up of a palisade-like layer of sterile, unicellular paraphyses having terminal hyaline appendages and fertile sporangia borne side by side. Both of these organs originate from the outer cells of the cortex of the sporophylls. As the sporangium matures, the cytoplasm draws away from the wall, leaving approximately 32 spores crowded tightly together. As these spores develop, the wall at the apex of the sporangium gradually becomes thickened, forming a cap-like structure. As the time approaches for the mature spores to leave the sporangium, they are seen to gradually emerge and float away. Contrary to the findings of other workers on the Laminariaceae, the spores do not seem to possess cilia or to be motile. It is repeatedly stated in the literature that the zoospores possess two lateral cilia of different lengths and that the "zoospores" swim rapidly away upon being released from the sporangium. Although several staining techniques were employed, no cilia were observed. Continued observation of material at different times of day and night failed to disclose any motility of the spores. The fact that the spores attach themselves to the slides in the culture dishes and germinate in the normal way indicates that they have sufficient buoyancy to float to a place of attachment.

The spore is spherical to ovoid, measuring 3 to 4 microns in diameter. When placed in the artificial culture solution, the spores float from 8-24 hours and then attach themselves to a surface. Germination begins and continues for several days while the spores enlarge to a diameter of 10-12 microns, secrete a distinct wall, and produce germ tubes. The chromatophore elongates and passes into the tube, usually dividing during the process of migration. When the plants are about a week old the first cross-walls appear, and several days later the majority of the germinating spores have the first wall across the germ tube.

THE GAMETOPHYTES.—For a period of time varying from a few days to several weeks, the plant remained in what may be called a temporary resting stage, the increase in size being very gradual. On the twentieth day some of the gametophytes had formed monosiphonous filaments from 3-5 cells in length, and by the end of the 36th day the plants were from 6-10 cells in length. By this time differentiation of the microgametophyte and megagametophyte occurred. The sex of the gametophytes could be distinguished by the size of the plant as a whole, by the dimensions of the cells, and by the difference in pigmentation. The microgametophyte (fig. 4) was smaller, with numerous branches that were short and compact or thinner and more elongated. The megagametophyte (fig. 6) was made up of large oval cells with many conspicuous chromatophores, and it branched less.

FORMATION OF THE ANTHERIDIUM.—The mature microgametophytes (fig. 3) form slender, irregularly branched filaments with the antheridia forming con-

spicuous clusters at the tips of the branches. The production of antheridia is limited largely to the apical region, and the cells of the main axis remain entirely sterile. Any cell may function as an antheridium, the end cells being first to become thus specialized. As the process of antheridial formation proceeds and the sperms are discharged, the male gametophyte gradually becomes invisible. Under favorable conditions the gametophytes have been found to have reached maturity and to have produced gametes in about 60 days after the germination of the spores. One of the first signs of approaching maturity of a culture is the appearance in it of a few empty cells after the antherozooids have broken out. The entire contents of the antheridium is used up in the formation of a single male gamete.

The escape of the antherozooids was not observed; therefore, whether the cells which emerged were free-swimming is still to be determined. The conclusion that they were sperms is evidenced by the presence in the cultures of the antheridial structures, by the occurrence of single small bodies in these structures, and finally by the empty cells from which the sperms had escaped.

FORMATION OF THE OÖGONIUM AND EGG.—In cultures 60 days old containing mature megagametophytes erect branches are commonly found bearing large apical cells (fig. 6) conspicuous on account of their dense contents. In older cultures, oögonia occur not only in the apical cells of the branches, but any cell of the filament may elongate at right angles to the main axis and become an oögonium. These cells elongate to about twice the length of the normal vegetative cells as the wall at the apex becomes swollen. The walls of the oögonial cells have a somewhat heavier appearance, the length of the cell is much augmented, and the apex is decidedly swollen. These features make the oögonial structures very conspicuous. At the apex of the oögonium the thickened wall splits, developing a beak through which the egg emerges. The egg rounds up as it emerges from the oögonium and usually remains in position at the tip, where it is fertilized. The oögonium contains, in addition to the egg, a small amount of residual cytoplasm and a few chromatophores. The unfertilized egg is a spherical or ovoid body surrounded by a delicate plasma membrane. It contains many chromatophores which are distributed irregularly throughout the cytoplasm. Although the egg usually remains at the mouth of the oögonium in *Eisenia arborea*, it is sometimes displaced and develops entirely away from the megagametophyte. There can be no doubt that there is here a differentiated oögonium and that the single egg produced by it is fertilized after emergence.

DEVELOPMENT OF THE YOUNG SPOROPHYTE.—The sporophyte shows two methods of development. In one the fertilized egg elongates somewhat and forms a transverse wall dividing the sporophyte into two unequal parts. At first, cell division takes place in one plane only, resulting in a short thick mono-

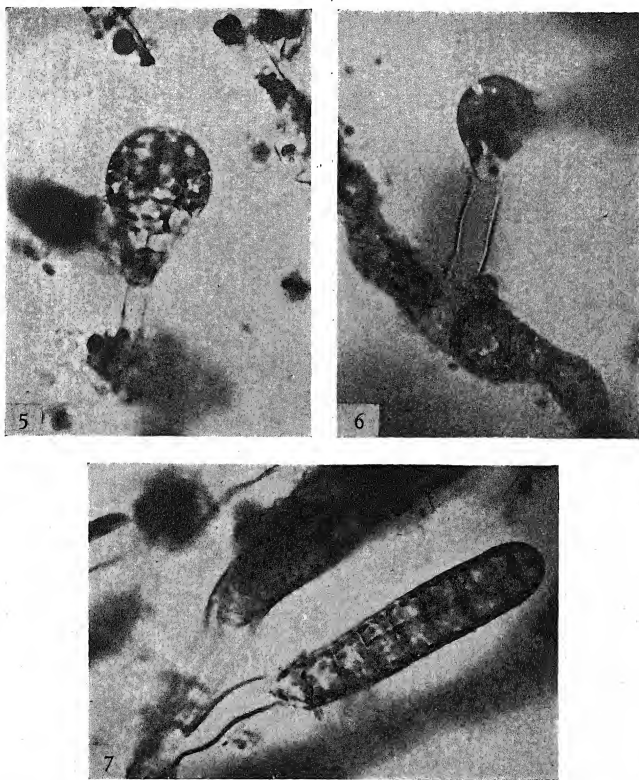


Fig. 5-7. *Eisenia arborea* Aresch. Plants grown upon slides. $\times 1000$.—Fig. 5. Young sporophyte with oogonium at base.—Fig. 6. Megagametophyte with oogonium and megagamete.—Fig. 7. Young sporophyte with oogonium at base.

siphonous filament six to twelve cells in length (fig. 7). The cells in the apical region are the first to divide by a longitudinal septum and are followed rapidly by the other cells of the filament, with the exception of the basal cell which begins to elongate. The other method of development results when the oogonium develops between two cells in the body of the megagametophyte, and in this case the fertilized egg shows cells dividing in more than one plane (fig. 5). The sporophytes are not attached to the oogonium by the basal cell but are held in position by the gelatinous matrix which surrounds the egg. The majority of the

new plants are held to the empty oogonium, but a few are found floating. The floating sporophytes do not seem to do so well or to develop so rapidly as those remaining on the plant.

SUMMARY

In *Eisenia arborea* Aresch. the massive macroscopic sporophyte alternates with the microscopic gametophyte generation.

Spores are morphologically homosporous but physiologically heterosporous, and they are probably non-motile and arise from unilocular sporangia.

Microgametophytes are smaller than the megagametophytes and produce antheridia each with a single sperm in clusters at the tips of the lateral branches.

Megagametophytes are larger than the microgametophytes and produce apical or intercalary oogonia

within the body of the plant. The egg is largely extruded and apparently soon fertilized.

The young sporophytes are monostromatic.

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INFLUENCE OF AUXINS ON THE GROWTH OF CHLORELLA VULGARIS¹

Robertson Pratt

ALTHOUGH SOME evidence has been published which indicates that heteroauxin and perhaps other plant hormones are capable of promoting cell division in plants (Jost, 1935; Snow, 1935), the literature contains few quantitative data on this phase of auxin activity. This paper gives quantitative evidence which clearly shows that under suitable conditions several synthetic chemical compounds promote cell multiplication in the unicellular green alga *Chlorella vulgaris*.

METHODS.—Cells for the experiments were withdrawn from rapidly growing four-day-old stock suspensions of *Chlorella vulgaris* cultured as previously described (Craig and Trelease, 1937; Pratt and Trelease, 1938). The cells were separated from the culture medium and were washed in three changes of distilled water by repeated centrifugation and decantation. They were then suspended in a suitable

quantity of distilled water, and uniform volumes of the suspension were pipetted into 500 cc. Florence flasks, each of which contained 150 cc. of nutrient solution (Craig and Trelease, 1937). In addition to the standard nutrient salts the solution in some of the flasks contained different concentrations of indole-3-acetic acid, indole-3-n-butyric acid, indole-3-n-propionic acid, potassium acetate, glucose, acetic acid, glycine, or allantoin. In the first experiment no attempt was made to control the initial pH of the solutions, but in the second and third experiments initial pH values of 4.0-4.5 were obtained by the addition of H_2PO_4 and KOH. The initial pH of the unadjusted standard nutrient solution was 4.4. Measurements were made with a Beckman glass-electrode pH meter.

The cultures received light from a north window. All the cultures in a given experiment were uniformly exposed to the same fluctuations in light and tempera-

¹ Received for publication April 4, 1938.

ture. Seasonal variations occurred, however, and these probably account, in part at least, for some of the quantitative differences between the results of the different experiments. No artificial illumination was used, nor was additional CO_2 supplied. The temperature was approximately $18-22^\circ\text{C}$.

Estimates of the cell population were made from haemocytometer counts at the beginning of the experiments and at suitable subsequent intervals. Since each flask initially contained 150 cc. of culture solution, the volume of the liquid was not greatly altered by the withdrawal of the small samples required for making cell counts. An ocular micrometer was employed to measure cell diameters.

RESULTS OF EXPERIMENTS.—Cell number.—The most prominent result of these experiments was the great increase in the population of cultures that were supplied with suitable concentrations of the synthetic growth hormones (fig. 1-2). The experiments furnish clear evidence that the three synthetic auxins employed were capable of increasing cell multiplication in *Chlorella*. Indole-3-acetic acid (heteroauxin) appeared to be the most effective of the compounds tested. Best growth always occurred in solutions that contained 50 ppm. ($2.85 \times 10^{-4}\text{M}$) of this compound.

The cultures that contained 10 ppm. or 50 ppm. of the growth substances tested could always be easily

distinguished from the controls by the darker green color due to denser population. The cultures that received 100 ppm. of heteroauxin were distinctly greener than the control but had a somewhat yellowish tinge. No growth occurred in solutions that contained 200 ppm. ($1.14 \times 10^{-3}\text{M}$) of heteroauxin.

The increased growth that was observed in the heteroauxin cultures cannot be attributed to variation in the hydrogen-ion concentration, since addition of the weakly ionized growth-promoting compounds produced relatively little change in the initial pH value of the nutrient solution. The values ranged from pH 4.4 (control solution) to pH 4.0 (200 ppm. heteroauxin).

The effectiveness of different synthetic indole-compounds was compared by using indole-3-acetic acid, indole-3-n-propionic acid, and indole-3-n-butyric acid,² each being separately tested in concentrations of 10 and 50 ppm. Three of the seven cultures became contaminated with fungi after about two weeks, so that it was impossible to obtain final values for the cultures in those flasks. Enough data are available, however, to indicate that for both 10 ppm. and 50 ppm. the descending order of effectiveness in promoting cell multiplication in *Chlorella vulgaris* is indole-3-acetic acid, indole-3-n-propionic acid, indole-3-n-butyric acid. This is shown in table 1.

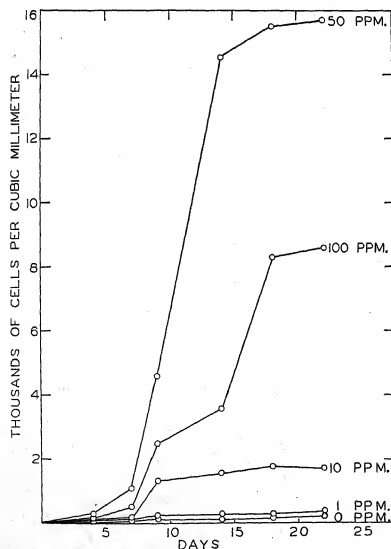


Fig. 1. Growth of *Chlorella vulgaris* in nutrient solutions containing different concentrations of indole-3-acetic acid (heteroauxin).

TABLE 1. Growth of *Chlorella vulgaris* in solutions containing different auxins.

Substance	Concentration	pH	Cells per c.mm.
Indole-3-acetic acid	10 ppm.	4.4	1900 ^a
" " " "	50 "	4.2	7900 ^a
Indole-3-n-propionic acid	10 "	4.2	1600 ^a
" " " " " "	50 "	4.1	4700 ^b
Indole-3-n-butyric acid ..	10 "	4.4	750 ^b
" " " " " " ..	50 "	4.2	4500 ^a
Control	—	4.4	400 ^a

^a Population after 26 days. There was very little, if any, growth after that time.

^b Population after 15 days. Growth was not completed at that time, but the culture was discontinued because it had become contaminated.

Some other carbon-containing compounds were tested to see whether or not they would give results similar to those obtained with the indole compounds. Solutions containing 50 ppm. and 100 ppm. of indole-3-acetic acid contain approximately 34 ppm. and 68 ppm., respectively, of carbon. Although it seemed unlikely that such small amounts of carbon would furnish sufficient energy to account for the observed increase in growth, an experiment was performed in which similar amounts of carbon were supplied as potassium acetate, glucose, acetic acid, glycine, and

² These substances were obtained from the Eastman Kodak Co. They were not recrystallized or otherwise purified.

allantoin.³ The final values obtained are given in table 2, and some of the data are plotted in figure 2. The results indicate that the indole-compound exerted some specific influence, since none of the other substances tested was as effective as heteroauxin in promoting cell division. The heteroauxin did not

TABLE 2. Growth of *Chlorella vulgaris* in solutions containing different carbon compounds.

Substance	Concentration of carbon, ppm.	pH ^a	Final number of cells per c.mm.
Control	0.0	4.4	260
Indole-3-acetic acid	137.2	4.0	No growth
" " " "	68.6	4.1	5,610
" " " "	34.3	4.2	11,500
" " " "	6.9	4.4	2,010
" " " "	0.7	4.4	595
Potassium acetate .	68.6	4.3	4,910
" " " "	34.3	4.2	2,100
Glucose	68.6	4.3	3,120
" " " "	34.3	4.3	2,600
Acetic acid	68.6	4.0	890
" " " "	34.3	4.1	790
Glycine	68.6	4.5	650
" " " "	34.3	4.4	470
Allantoin	68.6	4.4	450
" " " "	34.3	4.4	390

^a In some instances the value was obtained by the addition of H_3PO_4 or KOH.

³ Allantoin has frequently been referred to as stimulating the healing of wounds in animals, presumably through the promotion of cell multiplication.

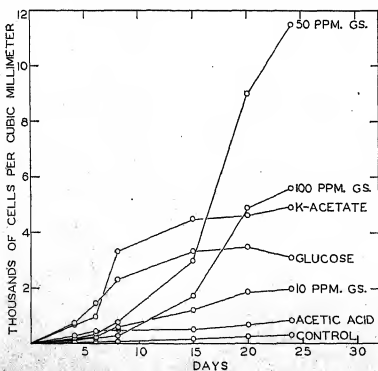


Fig. 2. Growth of *Chlorella vulgaris* in nutrient solutions containing indole-3-acetic acid (GS.), potassium acetate, glucose, or acetic acid. The carbon concentration in the potassium acetate, glucose, acetic acid, and 100 ppm. heteroauxin solutions was approximately 68 ppm.

serve, like glucose, merely as a source of readily available energy. Nor was either potassium acetate or acetic acid particularly effective in increasing the growth of *Chlorella*.

Cell size.—Heteroauxin promotes cell enlargement in many higher plants, and Yin (1937) reported that the mean diameter of *Chlorella vulgaris* cells became about twenty per cent greater when heteroauxin in suitable concentration was added to the nutrient solution. In the present work, however, heteroauxin, in concentrations ranging from 0 to 100 ppm., produced no significant change in cell diameter (table 3).

TABLE 3. Mean cell size of *Chlorella vulgaris* in solutions containing different amounts of indole-3-acetic acid (heteroauxin).

Concentration of heteroauxin ppm.	Mean cell diameter in microns ^a	
	Exp. I (22 days)	Exp. II (24 days)
0	5.49 \pm 0.12 ^b	5.63 \pm 0.11
0.1	5.46 \pm 0.12	—
1	5.40 \pm 0.09	5.27 \pm 0.13
10	5.15 \pm 0.13	5.54 \pm 0.13
50	5.51 \pm 0.15	5.51 \pm 0.09
100	5.39 \pm 0.12	5.71 \pm 0.13
200	No growth	No growth
Glucose ^c	—	5.40 \pm 0.09
K-acetate ^c	—	5.35 \pm 0.11

^a Figures are based on measurements of approximately 250–300 cells for each concentration. All the cells in a given area of the microscope field were measured.

^b The precision given is the probable error.

^c Supplying 68 ppm. carbon.

In these experiments heteroauxin was effective in promoting cell multiplication in *Chlorella vulgaris*, but it had exerted little, if any, influence on the cell size at the end of approximately three weeks. The cultures were past the grand period of growth at that time, and the populations were increasing very slowly, if at all.

SUMMARY

Chlorella vulgaris was grown for about three weeks in nutrient solutions to which were added different synthetic chemical growth-substances.

Cell multiplication was markedly increased in the presence of 50 ppm. indole-3-acetic acid (heteroauxin), indole-3-n-propionic acid, and indole-3-n-butyric acid. Concentrations of 10 ppm. also caused appreciable stimulation. The acetic acid compound was most effective, and the butyric acid compound least effective at both concentrations tested.

The observed effect was not the result of variation in the initial pH value of the solutions.

The indole compounds apparently exerted a specific effect on the growth of *Chlorella* and did not, like glucose, act merely as sources of readily available carbon. The increase in cell number brought about

by glucose was relatively small in comparison with that produced by heteroauxin.

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FACTORS AFFECTING THE REDUCTION OF SILVER NITRATE BY CHLOROPLASTS¹

Eliot Weier

LOEW and Bokorny (1882), apparently, were the first to notice the power of living material to reduce silver nitrate. In 1918 Molisch described the reduction of silver nitrate by living chloroplasts. He noted that dead chloroplasts, etiolated chloroplasts, leucoplasts, and many chromoplasts will not reduce silver nitrate. According to him the reaction will take place after the plants have been kept in darkness for four months. Gautheret (1934) maintains that at least several seconds of exposure are required for the reaction. Since Molisch, describing his experiment, does not state that the reaction was carried out in complete darkness, possibly enough light reached his plants during the operation to give positive results. Both Molisch and Gautheret agree that the reducing substance cannot be chlorophyll. Since only living chloroplasts can reduce the silver nitrate, Molisch concludes that the reducing substance may be somehow associated with photosynthesis; Gautheret that it must be a photolabile substance. Molisch and Gautheret disagree as to its nature. The former thinks that it may be either an aldehyde or a peroxide, although he does not believe that the case for either is definitely proved. Because of its precipitation by lead salts, Gautheret suggests an oxyflavon.

Meyer (1917) studied a substance which he thought was assimilated in the chloroplast and which reduced ammoniacal silver nitrate. It was destroyed at 48°C. but could be distilled in vacuum. It is not present in etiolated leaves or in fungi. He thought it to be a hexynaldehyde of the formula $\text{CH}_3-(\text{CH}_2)_2-\text{CH}=\text{CH}-\text{COH}$. Weier (1936) believes that an essential oil causes the reaction.

Giroud and his co-workers (1934, 1935; Giroud, 1935) noted a correlation between chlorophyll and ascorbic acid content of plants—that is, plants and plant parts containing chlorophyll show a higher ascor-

bic acid content (determined with 2-6 dichlorophenol-indophenol) than plants with no chlorophyll. Since both ascorbic acid and chloroplasts will reduce silver nitrate, these workers conclude that ascorbic acid is associated with the chloroplasts. They go on to point out a relation between chromoplasts and ascorbic acid. Dischendorfer (1937a, 1937b) and Mirimanoff (1938) question the accuracy of the conclusions of the Giroud school regarding the correlation between vitamin C content and the chromoplast pigments. Dischendorfer concludes that the chloroplasts elaborate vitamin C and that the grana are the centers of this elaboration. Mirimanoff states that the reduction takes place in the vacuole. Weber (1937a) accepts the hypothesis that the chloroplasts are the carriers of vitamin C, although doubting that the grana are the only structures involved. He (1937b) has recently reviewed the literature dealing with the Molisch reaction. The present paper contributes additional facts regarding the conditions under which the Molisch reaction takes place, and compares the properties of the substance responsible for it with some properties of ascorbic acid. The reaction was studied under three fundamentally different conditions: (1) living leaves treated directly with silver nitrate solution; (2) leaves killed in air and then treated with silver nitrate; (3) leaves killed in the absence of oxygen and then treated with silver nitrate.

PRELIMINARY TESTS WITH ASCORBIC ACID.—Although the properties of ascorbic acid are well known, our work required some preliminary experiments on conditions controlling its oxidation, particularly with reference to silver nitrate. The important points learned from these experiments may be briefly summarized: Ascorbic acid, in pure aqueous solution, is rather more stable toward heat and oxygen than a survey of the literature would lead one to believe. Similarly, the oxidation of methylene blue requires a prolonged exposure to intense light, though in all probability the presence of certain cell constituents may materially accelerate this reaction. The rate of oxidation of ascorbic acid is, of course, greatly affected by the hydrogen-ion concentration of the solution. This is particularly noticeable with silver nitrate, the

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Conducted in the laboratory of the Division of Plant Biology, Carnegie Institution of Washington, Stanford University, California.

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reduction of silver nitrate by ascorbic acid being much more rapid in solutions of pH 6 and 7 than in solutions of 3 and 4. It is essential to consider these facts in all histochemical work on ascorbic acid.

NORMAL PLASTID STRUCTURE.—Material for the present observation was gathered from clover plants in the greenhouse. In all leaves from plants growing in full sun the chloroplasts were homogeneous. The granular structure recently described by Doutreligne (1935), Heitz (1936), and Weier (1936) was found only in such plants as had been shaded for 15 hours before they were used for experimentation. It has been definitely demonstrated that both types of chloroplasts are normal (Weier, 1938).

LEAVES KILLED DIRECTLY IN SILVER NITRATE.—*Clover leaves.*—For comparison, free-hand sections of leaves grown in shade and sun were cut directly in a 2 per cent solution of silver nitrate. These sections varied in thickness from one to several cells. Leaves of plants taken from sun and shade were cut into strips approximately 4 mm. wide. These were dropped into the 2 per cent silver nitrate solution, and rapid infiltration was obtained by evacuation.

Silver nitrate was reduced in the form of small irregular granules, which were more or less evenly distributed throughout the plastid when leaves grown in full sun were sectioned directly in 2 per cent silver nitrate solution (fig. 1).

When pieces of leaves from similarly illuminated plants were impregnated with silver nitrate in vacuum and then sectioned, the stroma of some plastids had become a dark tan or brown, while several clear, round spaces were evenly distributed throughout this background (fig. 2). More commonly after this treatment the chloroplasts were capped, either on one side or along one edge, with an irregular, crescent-shaped, densely granular mass of silver (fig. 3). This mass shaded off abruptly into the plastid proper, which had usually become impregnated with sufficient silver to be a dark tan. The outer edge of the blackened mass, which was several micra in thickness, was fairly sharp in contrast to the more ragged and diffuse boundary between the plastid and the precipitate (fig. 3). It was difficult to decide whether the reaction had taken place within the chloroplast or at the boundary between the chloroplast and cytoplasm. There was no perceptible reaction in other regions of the cell under these conditions.

A similar cap of silver, though much denser, more homogeneous, and sharper-bordered on both sides, was observed when leaves that had been in the dark or shade for several hours were impregnated with silver nitrate in vacuum. Most chloroplasts subjected to this treatment were a homogeneous brown, with a narrow band of precipitate completely or almost completely surrounding them. A very distinct line separated that region of the plastid where the silver nitrate was heavily reduced from the larger brown region. In all sections it appeared as a rim or a partial rim around the plastid. Focusing did not give the impression of a shell completely surrounding the

chloroplast; yet if only a rim or a narrow region of the plastid reduced the silver nitrate, one might expect that a few plastids would present to view a heavy line of silver running along one face. This was never noted. Reduction of the silver nitrate was not confined to the chloroplasts in shaded leaves impregnated with silver nitrate by vacuum. In many sections irregular granules of silver were found scattered throughout the tissue—in the cytoplasm, the vacuoles, and even in the intracellular spaces.

The study of living chloroplasts sometimes gave the impression that each starch grain was surrounded by a separate vesicle of chlorophyll-impregnated cytoplasm. A few very distinct pictures supporting this concept were obtained in the silver nitrate impregnation of leaves from plants grown in the sun. The whole chloroplast was tan. A very lightly impregnated region of cytoplasm surrounded the whole body. The starch grains within it were each surrounded by a mass of cytoplasm staining more densely than that surrounding the composite structure (fig. 4).

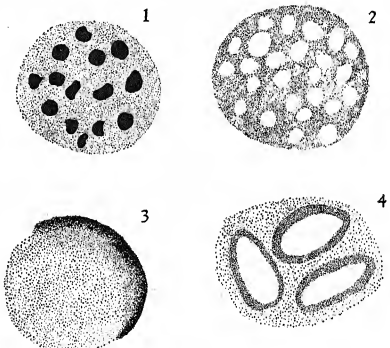


Fig. 1-4. All drawings refer to the reduction of 2 per cent silver nitrate by chloroplasts of clover leaves.—Fig. 1. Leaves grown in full sun. Pieces of leaf impregnated in vacuum. Silver nitrate reduced in irregular granular clumps.—Fig. 2. Leaves grown in full sun. Sectioned directly in silver nitrate. Stroma brown; grana colorless.—Fig. 3. Leaves grown in full sun. Sectioned directly in silver nitrate. Reduction along one side of chloroplast.—Fig. 4. Leaves grown in full sun. Sectioned directly in silver nitrate. Each starch grain is surrounded by a sheath of lightly impregnating cytoplasm.

The stroma occasionally stained an even, light tan. This color was noted when leaves exposed to full sun were sectioned directly in silver nitrate and when pieces of leaf were killed by chloroform fumes in an atmosphere of hydrogen, as will be described later. In this latter case a heavy black precipitate of silver in the form of irregular rods, granules, hollow spheres, and large masses appeared in the cytoplasm.

In cells bordering the free-hand sections, where presumably some injury had occurred, or in regions where the sections were only two or three cells thick, there was usually no reduction of the silver nitrate. Since cells thus situated will usually accumulate neutral red, the inference is that these cells were alive; but through diffusion or oxidation of the reducing substance or through some other factor the silver nitrate was not reduced.

Chloroplasts sectioned by the razor or isolated in the silver nitrate solution did not reduce silver nitrate. These plastids also showed typical necrotic changes (Weier, 1938). This fact, together with the lack of reduction in cells close to the edge of the section, indicates the delicate balance existing between the chloroplast and its environment.

Sorrel and oxalis leaves.—Sorrel and oxalis leaves were brought in from the lath house on an overcast morning. The pH of their cell sap was less than 2. The chloroplasts in leaves sectioned directly in sugar solution contained grana to which the chlorophyll was confined. In leaves sectioned directly in 2 per cent silver nitrate there was some reduction of the silver along the cell walls but no blackening of the chloroplasts. When small pieces of leaf material were impregnated with silver nitrate by vacuum, the cut edges of the sections became brown. Microscopic observation showed this browning to be confined to the chloroplast. Clover leaves treated in the same manner and at the same time strongly reduced the silver.

As determined by the 2:6 dichlorophenolindophenol test, both sorrel and oxalis leaves contain appreciable amounts of ascorbic acid. The negative or faint Molisch reaction, however, is easily explained; the low pH values occurring in the cell sap of these leaves prevent the reduction of the silver nitrate by the ascorbic acid.

Etiolated barley seedlings.—Small amounts of ascorbic acid have been detected in many etiolated seedlings. Molisch (1918) reported that such material did not reduce silver nitrate. Our studies on etiolated plants have not been extensive, but a very definite, though faint, Molisch reaction has been obtained with etiolated barley leaves. Pieces of leaf were impregnated in vacuum with 2 per cent silver nitrate. Macroscopically the leaf pieces were grayish. Sections examined under the microscope showed some half-dozen clear black granules to be present in each chloroplast, as in figure 1.

Lemon, pineapple, and potato.—Ascorbic acid is present in large quantities in the eatable pulp and rind of lemons, and a positive test for its presence was obtained in sections of fresh pineapple and potato. Pieces of pineapple and potato placed in 2 per cent silver nitrate solution reduced the silver nitrate slowly, but eventually a heavy black precipitate formed.

Extracts and sections of the eatable, juicy pulp of lemons did not reduce silver nitrate, but an intense black precipitate formed in the rind. This would be the result expected if ascorbic acid were responsible

for the Molisch reaction. The acid condition of the pulp would prevent the reduction of the silver nitrate, whereas in the more alkaline rind it should be intense.

LEAVES KILLED IN AIR AND TREATED WITH SILVER NITRATE.—As Molisch (1918) has pointed out, chloroplasts in living cells only are able to reduce silver nitrate. In studies on the structure of the chloroplasts (Weier, 1938) the silver nitrate reaction was used as one of the several tests for the living state. About 15 seconds' exposure to chloroform fumes, 30 seconds to toluene, and about 1 minute to formaldehyde sufficed to render the leaves incapable of reducing silver nitrate, of accumulating neutral red, and of hydrolyzing starch.

Clover leaves were cut in sections and placed in dry test tubes. The tubes were held for 5 to 10 minutes at temperatures ranging from -50°C . to -30°C . and at 100°C . and were then allowed to come to room temperature. Silver nitrate was poured over the leaf sections and impregnation was obtained by evacuation. No reduction of silver nitrate occurred. An aqueous extract prepared from these leaves did not reduce silver nitrate. The indophenol test indicated the absence of ascorbic acid from leaves killed in air by these temperatures.

Leaves were killed by placing them in boiling water and holding them at that temperature for several minutes. These leaves were able to reduce silver nitrate, and the extract rapidly discolored indophenol, showing that the ascorbic acid had not been destroyed.

Clover leaves were killed by placing sections in 8 per cent acetic acid. They were washed in water to remove most of the acid and tested at once with silver nitrate. There was no immediate blackening of the leaf, but upon standing, the cut edges of the leaf became brown as the sorrel and oxalis leaves had done. Leaves killed in acidified silver nitrate behave similarly. Microscopic examination showed the reduced silver nitrate well distributed throughout the leaf and not confined to the chloroplasts. The extract of leaves killed in 8 per cent acetic acid did not reduce silver nitrate. A white precipitate, presumably caused by the chlorides present in the leaf, formed when silver nitrate was added. Upon standing, this precipitate turned brown.

The extract obtained by killing and grinding leaves in 8 per cent acetic acid rapidly reduced the indophenol. This extract would not be expected to reduce silver nitrate, as ascorbic acid cannot do so in an acid medium.

LEAVES KILLED IN AN ATMOSPHERE OF HYDROGEN AND TREATED WITH SILVER NITRATE.—Tissues killed in air by any of several different methods no longer reduce silver nitrate. The substance that reduces the silver nitrate must have been oxidized by the oxygen of the air at the moment of cellular death. The question now arises as to how this substance would be affected if the leaves were killed in an atmosphere free of oxygen. Would it still reduce silver nitrate even though the cells were no longer living? Ascorbic

acid should not be oxidized when tissues are killed in the absence of oxygen. The presence or absence of the Molisch reaction after this treatment should further help to identify the substance responsible for this reaction with ascorbic acid.

Clover leaves were cut into strips about 5 mm. wide. The experiments were carried out in a large glass tube fitted with glass stopcocks and with a manometer so that it could be evacuated and flushed with hydrogen repeatedly. The stopper of the large tube was also fitted with a dropping funnel so arranged that the anesthetic could be run into a separate vial at the bottom of the tube and that subsequently silver nitrate solution could be run on to the leaf strips.

In an experiment the tube was evacuated and hydrogen was admitted slowly until atmospheric pressure, as indicated by the manometer, was reached. Several minutes were allowed for the diffusion of hydrogen into the leaf and for the displacement of as much residual air as possible. The tube was then again evacuated, and hydrogen admitted a second time. While a small negative pressure still existed, 2 to 5 cc. of chloroform were run into the vial. A small positive pressure was then developed, and the apparatus was so arranged that hydrogen flowed slowly through it. Leaves were treated with chloroform fumes from 2 to 20 minutes. At the end of this time the pressure in the large tube was slightly reduced, and a small amount of 2 per cent silver nitrate was run over the leaf strips. Rapid penetration of the silver nitrate was obtained by immediate evacuation of the tube and readmission of hydrogen thereto. Treatment with silver nitrate lasted 15 to 20 minutes. This time was evenly divided between an atmosphere of hydrogen and a vacuum.

Contrary to results with leaves killed in air, when the leaves were killed in an atmosphere of hydrogen and then treated with silver nitrate in an atmosphere of hydrogen, the reaction was definite and positive. Its intensity, however, depended upon the length of time the leaves were allowed to remain in the anesthetic. Leaves exposed to the vapors of chloroform for 2 to 10 minutes blackened uniformly along the edges of the strips. With 20-minute exposure there was little blackening, and 30 minutes in the chloroform fumes reduced the reaction to a faint browning of the leaf edges, indistinguishable from that resulting when sections were treated with silver nitrate after having first been killed by chloroform in the air. The 15 and 20-minute treatments showed considerable variation in the blackening, indicating an individual variation in the reaction of different leaves to chloroform treatment.

Microscopically, the cells killed by chloroform fumes in an atmosphere of hydrogen appeared quite different from those killed directly in the silver nitrate in air. All cells, including those treated for as short a time as 2 minutes, had the typical appearance of dead cells. The edges of the chloroplasts were crenate, and their borders were difficult to distinguish. The starch

grains, however, were not so apparent as in the chloroplasts of cells killed in air.

In all the hydrogen experiments, regardless of the length of time the leaf strips were exposed to chloroform fumes, the plastids themselves colored an even brown. The silver responsible for the macroscopic blackening was not located within the chloroplast. Black irregular granules were scattered irregularly throughout the cell and intercellular spaces. They were found most frequently in the cytoplasm but also in the vacuole and between the cells.

Ascorbic acid is easily extracted from leaves killed in similar manner. There is no reason to doubt that it exists within the tissue or that, after death of the cells and consequent destruction of semipermeability, it would diffuse throughout the tissue.

There is a general lowering of the pH values in dead tissue. If ascorbic acid is the substance responsible for the Molisch reaction, one would expect that as tissues become more acid, the reduction of silver nitrate within them would become less energetic. This result was obtained.

Leaf strips killed with toluene in a manner similar to those killed with chloroform gave analogous results except that the time of exposure had to be increased. After 30 minutes' treatment with toluene fumes in an atmosphere of hydrogen, the cells were dead, but the tissue could still reduce the silver nitrate. The extract obtained from these leaves gave tests for ascorbic acid.

Leaf strips were frozen at temperatures ranging from $-20^{\circ}\text{C}.$ to $-50^{\circ}\text{C}.$ In these experiments the temperature was maintained from 6 minutes to 2 hours, the leaf strips being kept in an atmosphere of hydrogen continuously. Thirty minutes was allowed for the tissue to warm up before the silver nitrate was added. Leaves subjected to the freezing temperatures for 30 minutes, with an additional half hour allowed for warming up, were variable in the reduction of silver nitrate. This was similar to the variation in the material treated for 20 minutes with chloroform. If there was a 2-hour interval between the 30-minute cold treatment and the addition of the silver nitrate, there was no reduction within the leaf itself, but a black precipitate formed in the silver nitrate solution. This observation indicates the solubility and diffusion of the reducing substance in water.

One gram of leaf material was killed by freezing in an atmosphere of hydrogen. Thirty minutes was allowed for the material to come to room temperature. Hydrogen flowed through the test tube during this time and while the strips were being extracted with 10 cc. of distilled water for 30 minutes. The tube was then opened to air, and 10 cc. of 2 per cent silver nitrate solution was added. Some blackening of the edges of the strips took place, and a copious black precipitate formed in the extract.

Similar results were obtained by freezing the leaves in a vacuum of about 20 mm. pressure. The leaf material was left in the freezing mixture for six minutes, allowed to thaw, extracted with 10 cc. of dis-

tilled water for 30 minutes; then 10 cc. of 2 per cent silver nitrate was added. There was some blackening of the strips, and a heavy black precipitate formed in the extract.

Leaves subjected to freezing treatment in air no longer reduced silver nitrate or indophenol reagent. When the killing took place in an atmosphere of hydrogen, the blackening was as intense as when the strips were killed directly by impregnation with silver nitrate. The extract of such leaves also contained large amounts of ascorbic acid.

The results of the present investigation are summarized in table 1. They indicate that the reduction

chloroplast; yet this fact is not definitely established. Possibly the vitamin is contained in all parts of the cell, the special conditions obtaining within the chloroplast bringing about the reduction of silver nitrate within or close to that body. Spoehr (1911), for instance, has brought forward evidence that the pH of the chloroplast may be of a higher value than that of the remainder of the cytoplasm. If this be the case, one would expect a more energetic reduction of silver nitrate in and around the chloroplast than in other parts of the cell.

Mirimanoff (1938) questions the reliability of acidified silver nitrate as an indicator for the presence of

TABLE 1. *Reduction of silver nitrate by chloroplasts.*

Plant	Method of killing	Molisch reaction	Presence or absence of ascorbic acid	Remarks
Clover leaves	Directly in silver nitrate	+	Present	
Clover	In 8% acetic acid	—	Present	Ascorbic acid reduces silver nitrate slowly in acid solution
Clover	Chloroform, toluene, formaldehyde	—	Absent	
Clover	-50°C. to -30°C.	—	Absent	
Clover	100°C. (Dry)	—	Absent	
Clover	In boiling water	+	Present	
Clover	Chloroform, toluene in hydrogen	+	Present	Molisch reaction becomes fainter as tissue became more acid
Clover	-50°C. to -30°C. in atmosphere of hydrogen or in vacuum	+	Present	
Sorrel and oxalis leaves	Directly in silver nitrate	—	Present	Ascorbic acid reduces silver nitrate slowly in acid solution
Etiolated barley seedlings	Directly in silver nitrate	+	Present	Molisch reaction faint
Lemon pulp	Directly in silver nitrate	—	Present	Ascorbic acid reduces silver nitrate slowly in acid solution
Lemon rind	Directly in silver nitrate	+	Present	Rind is not so acid as pulp
Potato	Directly in silver nitrate	+	Present	Molisch reaction slow in appearing
Pineapple	Directly in silver nitrate	+	Present	Molisch reaction slow in appearing

of silver nitrate may be explained by the presence of ascorbic acid in the cells of the leaf.

DISCUSSION.—In most instances the reduction of silver nitrate was confined to the chloroplast; but in some experiments, notably those carried out with tissues with a pH of 2 or lower, or after treatment with 8 per cent acetic acid, the reduction was fairly uniform throughout the cell. These results tend to indicate that the ascorbic acid is localized in the

ascorbic acid in plant tissues. He points out that tannins will reduce silver nitrate. Furthermore, silver nitrate is never reduced in or near the chromoplasts, and in tissues rich in ascorbic acid the reduction is frequently irregular. This corresponds with our experience with acidified silver nitrate and may be explained by the inhibitory effect of the low pH on the reduction of silver nitrate by ascorbic acid. Mirimanoff thinks that ascorbic acid cannot be responsible

for the reaction, because the reduction is less intense in plants which have been kept in darkness previous to impregnation with silver nitrate than in those which have been fully exposed to light. However, this result will be expected if ascorbic acid were responsible for the reduction. Many plant tissues become more acid in darkness (Richards, 1915). The reduction of silver nitrate should thus be less energetic in plants which have been kept in darkness previous to the application of silver nitrate. This would be even more true if an acidified silver nitrate were used in the test.

Frey-Wyssling (1937) described the sub-microscopic structure of the chloroplast as being a series of layers of protein and lipoidal materials. The chlorophyll is adsorbed in a monomolecular layer on the lipoidal strata. The stratification of protein and lipid material accounts for the birefringence exhibited by the chloroplast. Such a model is static and does not take into account the fact that the living chloroplast is an exceedingly active body in which many chemical changes of diverse nature are occurring at the same time.

Ascorbic acid is present in the living chloroplast. Unless proper precautions are taken, it is oxidized immediately upon the death of the cell. Once separated from the leaf tissue it is fairly stable. The conditions existing within the cell, which, upon death, cause the almost instantaneous oxidation of certain substances, are extremely interesting. They indicate the normal presence within the cell of strongly reacting substances which, though very close together, are separated in some manner so that if they do react, it is at a very slow and controlled rate.

Strain (1938) has found that the carotinoids are also oxidized rapidly when the cell is killed, and even chlorophyll is changed rapidly unless oxygen is excluded in the killing process (Mackinney, 1938). Strain (1938) has evidence for the presence in leaves of a very unstable substance which is easily oxidized and which apparently acts as a stimulus for the oxidation of the other compounds.

Spoehr's (1936) experiments on amylolysis also indicate the complexity of chloroplast structure. Hydrolysis of starch normally takes place in the living cell, whereas upon death amylolysis ceases even though neither enzyme nor starch has been destroyed or removed.

Any model of the sub-microscopic structure of the living chloroplast must be dynamic, similar to that of our present conception of the atom. It must show the presence of reacting substances within proximity, yet with their activity somehow controlled. These substances must be protected from oxygen, while free oxygen is the end-product of the principal reaction taking place within the chloroplast.

In other words, the chloroplast is a dynamic body; and lamina or fibrils, such as make up the cell wall, will scarcely give a complete picture of its sub-microscopic organization.

SUMMARY

Silver nitrate is reduced either as granules within the chloroplast, or uniformly along the edge of the chloroplast, or faintly throughout the stroma, or around the starch grains, or faintly throughout the cells of clover leaves, depending upon the treatment of the leaves previous to impregnation with silver nitrate.

Chloroplasts in leaves which have been killed in air by the fumes of toluene, formaldehyde, chloroform, by temperatures of $-50^{\circ}\text{C}.$ to $-30^{\circ}\text{C}.$ and $100^{\circ}\text{C}.$ will not reduce silver nitrate. As indicated by the dichlorophenolindophenol test, the ascorbic acid present in living clover leaves is oxidized or otherwise destroyed when leaves are killed in this manner.

Leaves killed in boiling water reduce silver nitrate, and the indophenol test indicates that the ascorbic acid has not been destroyed by this treatment.

Sorrel and oxalis leaves, with a cell sap of less than pH 2, and clover leaves which have been killed in 8 per cent acetic acid or in acidified silver nitrate either do not reduce silver nitrate or do so very faintly throughout the cells. Ascorbic acid is present in sorrel and oxalis leaves, and it is not destroyed in clover leaves when they are killed in 8 per cent acetic acid.

Pure ascorbic acid in a solution of pH 4 reduces silver nitrate very slowly, while reduction is energetic and rapid when the pH of the ascorbic acid solution is 7 or 8.

Silver nitrate reduction in etiolated barley leaves is positive, though very faint.

The eatable pulp of lemon and slices of pineapple, both giving an acid reaction and containing ascorbic acid, reduced silver nitrate very slowly. The more alkaline rind of lemon reduced silver nitrate very energetically.

Ascorbic acid is not destroyed when leaves are killed in an atmosphere of hydrogen. Such leaves and their extracts energetically reduce silver nitrate.

It is concluded that ascorbic acid is the substance responsible for the reduction of silver nitrate by the chloroplast.

The chloroplast is a dynamic body. Any model of its submicroscopic structure must show the presence of reacting substances in proximity, with their activity controlled, and the mechanism for this control, which is maintained only during life.

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A NEW CHYTRID ON NITELLA: NEPHROCHYTRIUM STELLATUM¹

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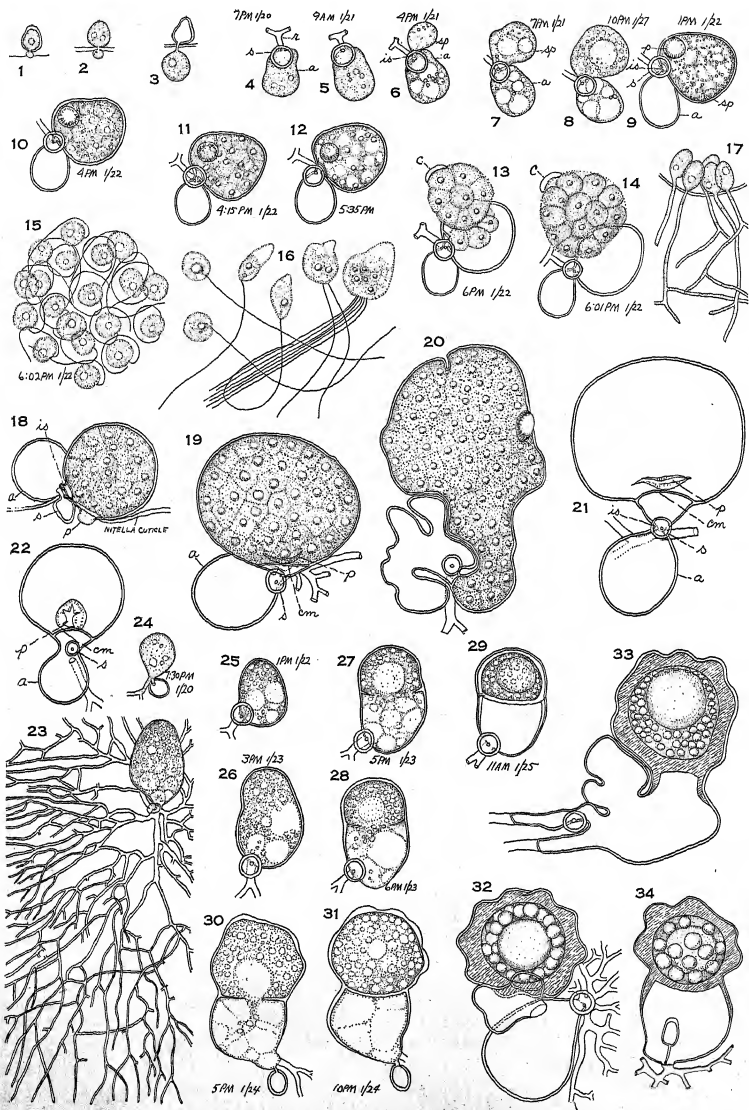
WHILE LOOKING for parasitic chytrids on *Nitella* I encountered in the unhealthy internodes a fungus with brown stellate resting spores and an unusual type of sporangial development. The fungus shows a rather striking relationship to Karling's (1938) new genus *Nephrochytrium*, and since the development of his fungus, though incompletely described, was rather unique, it seems of interest to describe another fungus with a similar course of development and to fill in certain details.

Karling selected the name *Nephrochytrium* for his new fungus because the zoosporangia and resting spores were somewhat kidney-shaped. In spite of the usual absence of kidney-shaped sporangia and resting spores in the present fungus, the general structure and development of the two are so similar that it seems advisable to include the present species in the genus *Nephrochytrium*. I am naming the present species *N. stellatum* because of the stellate resting spores.

Nephrochytrium stellatum sp. nov.—Thallus endophytic beneath the cuticle of *Nitella* or sometimes on inner side of cell membrane, monocentric. Each thallus consisting at maturity either of a richly developed

rhizoidal system, a more or less spherule apophysis and a zoosporangium, or of a resting body with rhizoids and apophysis. Sporangia roughly disc-shaped from pressure, usually circular in outline in face view but sometimes irregular in outline, particularly when growing with *Coleochaete*, usually with a basal columella which bulges into sporangium; 26-50 × 29-50 μ; with a basal, globose, ovoid, or sometimes lobed apophysis, the latter being connected to the sporangium by a narrow isthmus, apophysis 8.4-12.8 × 14.7-20 μ. The sporangium and apophysis, connected by the narrow isthmus, have an hour-glass-like appearance; the sporangium being much the larger part of the hour glass. Emergence pore formed near columella, usually not tubular but penetrating through the cuticle of the *Nitella*, bursting irregularly but always with a distinct cap. Spores emerging slowly in a spherical mass and remaining quiet for a few seconds at the sporangial mouth; spherical, about 5 μ thick, with a large refractive globule and a very long cilium, 35-40 μ long; zoospore case pyriform, becoming thick-walled, yellowish, and persisting on the surface of the *Nitella*, attached to the apophysis at the isthmus. Rhizoids elaborately developed, much branched, the ultimate branches with blunt tips, attached to the apophysis near the isthmus or more frequently to the isthmus by one main trunk. Resting spores near amber brown, nearly spherical but slightly wider than tall, usually with 8-12 large, rounded protuberances, rarely

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smooth, wall $2.8-4\mu$ thick, when ripe with one large oil (?) globule surrounded by a layer of smaller spherical bodies, $10-30\mu$ wide \times $11-29\mu$ long, usually $18-21\mu$ wide \times $16-19\mu$ long; spore with an empty, basal, usually barrel-shaped, or sometimes irregular apophysis, the walls of which are hyaline or nearly so; barrel-shaped part about $12-19\mu$ wide \times $8-15\mu$ long, but much smaller in depauperate specimens, wall about 2.5μ thick beneath the spore but thinning to a mere membrane at the basal rhizoidal end. The empty zoospore case becoming thick-walled and yellowish and remaining attached to the base of barrel-shaped part. Germination not observed.

Saprophytic in cells of *Nitella hyalina*, University Lake, Chapel Hill, N. C. Found on *Nitella* Jan. 7, 1938, after it had been in laboratory for several months. Usually occurring with *Coleochaete nitellarum* and occupying the same position just beneath the cuticle of the *Nitella*.

This is a very distinct fungus and may readily be separated from other chytrids by the structure and development of the zoosporangia and resting bodies. The present species may be distinguished from *N. appendiculatum* by the more or less disc-shaped sporangia connected with the nearly spherical apophysis by a narrow isthmus, the columella-like structure at the sporangial base, the irregularly shaped pore through which the spores emerge, and the stellate resting spores of the former. This genus would seem to belong in the Rhizidiaceae, as indicated by Karling (1938), close to *Diplophycitis*.

DEVELOPMENT OF THE ZOOSPORANGIAL THALLUS.—After a period of motility the zoospore comes to rest on the *Nitella* thread and assumes a pyriform shape with the broad base against the *Nitella* wall. The cilium disappears, the spore membrane thickens particularly at the narrow end, a distinct but transitory appressorium is formed, and penetration is effected by means of a fine tube which may be as much as 7μ long (fig. 1-3).

Although I have had an abundance of material, I have been unable to determine with certainty whether the apophysis or a rhizoid starts development first. Several instances have been observed of spores germinating within the old sporangial wall with the formation of rhizoids without an apophysis. Such spores failed to develop further than shown in figure 17. In the clearest instances observed, a small apophysis was visible before any rhizoids could be seen (fig.

2, 3). According to Karling (1938), in *Nephrochytium appendiculatum* the rhizoids branch several times before the apophysis begins to form. Also in *Rhizidiomyces apophysatus* (Zopf, 1884) and in a species of *Chytridium* (Couch, unpublished notes) the apophysis arises as a swelling in the basal rhizoid. In the present species, judging from the appearance of the mature structures, it seems that the apophysis might arise as a diverticulum of the primary rhizoid.

The rhizoidal system is elaborately developed (fig. 23). It consists of a main trunk which is attached to the apophysis very close to the isthmus, and from the main trunk arises a much branched system of delicate anastomosing threads. The threads do not taper at the ends, becoming as mere lines, but retain always a tubular appearance with blunt ends. The rhizoids of the present species are without the spindle-shaped swellings so conspicuous in *Cladochytrium replicatum*.

The time from spore germination to the maturity of the sporangium and the discharge of the spores is from two and a half to three days, as determined by observations on the development of five sporangia. The apophysis reaches mature size before the sporangium starts to develop. This is accomplished in slightly more than twenty-four hours. At first the cytoplasm in the apophysis is pale and hyaline with one or two small globules. As the apophysis grows, the globules increase in number and size, some being $3-5$ microns thick (fig. 3-5).

Three to six hours after the apophysis reaches mature size, the sporangium makes its appearance as a minute, hyaline, bud-like structure arising from the apophysis close to the point of union of the rhizoidal system and the apophysis (fig. 6, sp). The germ tube of the spore also connects with the apophysis in this same region, and one can readily see, even in the mature state, a minute pore indicating the connecting point. The empty, pale-yellowish, slightly thickened spore membrane persists here as in *Nephrochytium appendiculatum*.

As the sporangium grows, protoplasmic material from the apophysis flows through the narrow isthmus into the sporangium. About this time two or three vacuoles appear in the apophysis, and as the flow continues, the vacuolate condition becomes more conspicuous. Finally, by the time the sporangium has reached its mature size, the apophysis has become empty of its cytoplasm (fig. 6-9).

Fig. 1-34. All figures drawn with Zeiss water immersion objective and compensating oculars. Reduced one half in reproduction. All $\times 930$, except 22 and 23, which are $\times 580$.—Fig. 1-3. Early stages in spore germination.—Fig. 4-15. Stages in development of sporangium, spores, and spore discharge in the same sporangium. This is an exceptionally small sporangium. Fig. 4, 5. Old spore cyst, young rhizoids and apophysis. r, rhizoid; s, spore cyst; a, apophysis; sp, sporangium; v, vacuoles; p, emergence pore; c, cap; is, isthmus; cm, columella.—Fig. 6. Sporangium (sp) budding out from apophysis. In Fig. 10 note fusion of small globules to form large ones. For further explanation see text.—Fig. 16. Normal spores with one cilium and two abnormal spore masses.—Fig. 17. Spores germinating in sporangium. Note absence of apophysis. These spores failed to develop further than shown.—Fig. 18-22. Sporangia and apophyses of various shapes. Note star-shaped pore in fig. 22.—Fig. 23. Half mature resting body in which about one quarter of the rhizoids are shown.—Fig. 24-31. Stages in development of three different resting bodies; fig. 24, 25 represent one resting body; 26-29 another and 30, 31 a third.—Fig. 32, 33. Resting bodies with lobed and otherwise irregular basal parts.—Fig. 34. Typical resting body with empty barrel-shaped part and empty spore cyst attached.

At this stage the cytoplasm of the sporangium contains numerous vacuoles and a large number of refractive granules and globules of unequal size (fig. 9). As development goes on, the globules become smaller, more numerous, and more evenly dispersed through the cytoplasm (fig. 9). The globules now begin to unite in small groups to form the single globule of the spores. The development here differs somewhat from that found in *Rhizophidium globosum* (Couch, 1932). In that species the globules reach a fine state of dispersal and are all of about the same size, and the single globule of the spore is formed by the simultaneous fusion of several of the small globules. In the present species the globule in the spore is formed by the progressive fusion of globules of unequal size (fig. 10). Throughout this stage the protoplasm of the sporangium is conspicuously vacuolate. Also at about this time the emergence papilla first becomes evident on the sporangium. This structure is always formed close to the base of the sporangium near the old spore cyst and is first noticeable as a ring-like structure about as wide as, or sometimes wider than the old spore cyst. The emergence papilla becomes only long enough to penetrate the cuticle of the *Nitella*, and since this is formed on the part of the sporangium against the cuticle, the papilla is seldom more than a slight conical projection. The cytoplasm at this stage has the pale whitish gleam characteristic of chytid cytoplasm.

I have been unable to follow the stages in the cleavage of the protoplasm to form the spores. The cleavage seems to occur at the time when the small globules are fusing to form the single globule for each spore. After the spores have been formed, a number of small vacuoles appear which seem to be between the spores. These vacuoles increase in size and finally give the contents a net-work appearance (fig. 12). It is doubtless due to the swelling of the vacuoles that the emergence papilla is broken.

The immediate indication that the spores are going to emerge is the breaking off of the top of the papilla. This comes off in the form of a convex circular lid, but the mouth is almost always irregular in outline, being commonly slit-like or star-shaped. The lid, instead of remaining partly attached to the edge of the mouth, is completely broken off and pushed away some distance by the expanding spore mass (fig. 13).

The spores emerge rather slowly, forming a motionless, compact spherical mass at the sporangial mouth (fig. 14). In the compact mass it is possible to make out the individual spores, though they are polygonal in shape from pressure. After a very few seconds the mass spreads as though by the hydration of the gelatinous (?) matrix in which the spores are embedded. The spores separate from one another, assume a rounded shape, and at the same time the ellium uncovers from around each spore (fig. 15). Suddenly they begin to move, and more and more violently, until some break out of the spherical matrix, the others following in a few seconds. The entire process of emerging and dispersal lasts from one to three

minutes. Rarely, due to incomplete segmentation, double spores are formed, and on one occasion I observed a mass of protoplasm with six cilia and six oil globules (fig. 16). Such abnormal spore masses do not swim, but creep about in amoeboid fashion. I have never observed the germination of these abnormal spore masses. The normal spore is also capable of amoeboid motion and usually moves about in this fashion before settling down (fig. 16).

The mature and empty sporangium has a very characteristic and striking appearance by which one may immediately recognize this fungus. The most frequently observed shape with the characteristic slit-like emergence pore is shown in figure 21. Not infrequently, however, the apophysis and sporangium may not lie in the same radius, but the two may lie almost side by side, as in figures 18 and 19. When growing with *Coleochaete nitellarum*, the sporangium and apophysis may be very irregular in shape, due to pressure (fig. 20). As a rule the isthmus connecting the apophysis and sporangium is very narrow, but a few examples have been observed where the isthmus is 6-10 μ . wide (fig. 22). One unusual variation where the apophysis was not sharply separated from the sporangium was observed. Here the apophysis contained spores and thus functioned as part of the mature sporangium.

DEVELOPMENT OF RESTING BODIES.—The resting bodies are formed along with the zoosporangia. The spores which form both are apparently just alike, and the empty cysts of both are of the same size and shape and, since no fusions have been observed, the resting body does not seem to result from a sexual process. A reduced food supply may be one factor in causing the formation of the resting bodies, since developing resting bodies are more abundant than sporangia in old cultures, and furthermore, when a group of spores fails to disperse, all settling in one place, they develop into resting bodies instead of zoosporangia. However, since, as a rule, both structures develop under similar conditions and since the development of both is alike up to the formation of the apophysis, it would seem that some internal factor plays a part in determining whether a spore shall develop into a sporangium or resting body.

If the spore is going to form a resting body, the apophysis elongates upwards (fig. 24-26) instead of giving off a bud which enlarges to form the zoosporangium. Concurrently the protoplasm and fat globules concentrate in the distal half, the basal half becoming more and more vacuolate. After the resting body has reached mature size, a cross wall begins to form, separating the upper half from the lower part or apophysis (fig. 27-31). This wall is formed slowly and progressively from the side inwards. In one resting body the cross wall first became evident at 5 p.m., Jan. 23, 1938, and had extended all the way across at 6:30 p. m. the following day (fig. 27-29). The protoplasm continues to flow from the basal part through the central hole in the incompletely wall until the lower half is practically empty of its proto-

plasm. The globules of fat (?) now collect into a single large central or slightly eccentric body completely or partially surrounded by a layer of smaller globules, the protoplasm apparently occupying the spaces between the globules (fig. 29, 32-34). Meanwhile the wall of the spore has become wavy by the formation of several blunt protuberances. As the resting spore ripens, an exceedingly thick brownish inner wall is deposited. This is covered with the blunt protuberances on the outer side but is smooth interiorly. The wall of the empty part becomes thickened and brownish about halfway down the sides. As with the sporangia, the empty spore cyst persists here.

Ripe resting bodies have been in the laboratory for three months and have so far failed to germinate, in spite of freezing and drying.

Though there can be no question of relationship, there is a striking similarity between the development and structure of the resting bodies in this fungus and the azygospores in certain Mucorales—e.g., *Pilobolus namus*.

SUMMARY

A new species of the genus *Nephrochytium* is described. It is characterized by the globose or ovoid

apophysis connected to the somewhat flattened disc-shaped sporangium by a narrow isthmus, by the persistent pyriform spore cyst, which is also connected to the apophysis near the isthmus, by the slit-like or star-shaped emergence pore, and by the exceedingly thick-walled brownish resting body with large protuberances on the wall and the persistent empty basal apophysis and also the persistent empty spore cyst. Although the fungus grows as a saprophyte on *Nitella*, it has so far failed to grow on cooked *Nitella* or on the pollen grains of *Liquidambar* or *Pinus*.

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THE EFFECT OF LIGHT ON THE FRUITING OF MYXOMYCETES¹

William D. Gray

ALTHOUGH THE myxomycetes are unique in that they unite in themselves a number of characteristics of both animals and plants, they have been investigated by relatively few biologists. As a result of this neglect there are many gaps in our knowledge of their simpler physiological processes, and a search through the literature reveals the fact that little is known of them except perhaps the taxonomy within the group. This situation cannot be attributed to a lack of interest or inclination on the part of biologists, although it might be explained to some extent by the rather uncertain position of the myxomycetes. One reason for so little being known is probably the fact that only a few species have been cultured. Our present knowledge of the biology of this group has been obtained from the few forms which can be easily cultured in the laboratory and from observations made on species collected in their vegetative phase. The limitations of this latter procedure are obvious: No matter how assiduous a worker may be, there are many species which he may never see in the vegetative state; in fact, the plasmodia of only about one-third of the described species have ever been seen and recorded. The problem that immediately confronts us then is that of culturing species of myxomycetes which have never been grown in the laboratory.

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Certain species have been cultivated successfully, but these form a very small percentage of the total. *Physarum polycephalum* Schw. can be most satisfactorily cultured at present. Howard (1931) first successfully cultured this species in the laboratory, and further improvements by Camp (1936) made the cultivation of *P. polycephalum* much simpler than that of most fungi. Skupienski (1920, 1928) has cultured both *Didymium difforme* (Pers.) Duby and *D. nigripes* (Link.) Fr. on potato and carrot decoction agar, and Lister (1888) has described the cultivation of *Badhamia utricularis* (Bull.) Berk. on the sporophores of various agarics. The limitation of Lister's method is obviously the fact that such sporophores are not available at all times of the year. Klebs (1900) cultured *D. difforme* and *D. effusum* (synonym of *D. squamulosum* (Alb. & Schw.) Fr.), and Cayley (1929) cultured *D. nigripes* and *D. xanthopus* (Ditm.) Fr., in addition to the two species of *Didymium* used by Klebs, on Knop agar. A few other forms have also been cultured, but, considering the fact that there are about four hundred species of myxomycetes, it is clear that comparatively few have been grown in the laboratory.

Many attempts of the writer to cultivate various species (largely of the order Physarales) from living plasmodia, collected in their natural habitats, have generally failed because of the rapid formation of fruiting bodies by the plasmodia when brought into the laboratory, despite efforts to simulate conditions

under which they were growing in nature. Such results lead to the suggestion, already advanced by Cayley (1929) and Seifriz and Russell (1936), that there is a definite rhythmical fruiting exhibited by myxomycetes. Cayley found that *Didymium difforme* possesses a much shorter vegetative phase than either *D. nigripes* or *D. squamulosum* but stated that the factors causing plasmodia to form fruiting bodies are "still a complete mystery" (p. 234). If such rhythmical fruiting does exist, it is highly possible that the species observed by the writer may possess rhythms of relatively short duration in so far as the vegetative phase is concerned. Unless this rhythm is so innate as to be unalterable, it should be possible, by controlling various environmental factors, to vary the rhythm one way or the other so that plasmodia would vegetate for a longer or shorter period than normal. Thus it is entirely possible that a larger number of species might be brought into and maintained in cultivation if methods of prolonging the vegetative phase might be found. Of course, the problem of nutrition must also be considered, but even if the nutritional requirements of a species be known, it would be an extremely arduous task to maintain it in culture if it remained in the vegetative state for only a short period. Undoubtedly, a plasmodium about to form fruiting bodies differs fundamentally from one which is beginning its vegetative existence, even though the exact nature of the difference is not known. If the change from vegetative to fruiting phase is due to chemical factors, physical factors, or a combination of both, it should be possible to hasten or delay it. As mentioned above, the factor of nutritional requirements is an important one, and no attempt is made to minimize it; however, this can undoubtedly be ascertained by experimentation with various species.

The present series of experiments are concerned with the influence of light upon the length of time required to develop fruiting bodies in various species of myxomycetes. There is a possibility that these findings may have a bearing upon the culture of other species as well as contribute to our knowledge of the physiology of the group. It is quite probable that temperature, moisture, texture of substratum, nutrient materials, and degree of acidity or alkalinity of the medium are also important, but in the present work the influence of light alone is described.

Almost no investigations have been made of the effects of light upon myxomycete plasmodia. The most extensive work in this field was that Baranetzki (1876), who, however, limited himself almost entirely to studies of the heliotropic responses of plasmodia of *Fuligo septica* (L.) Weber and the interrelationships of heliotropic and geotropic responses. The remaining references in the literature are merely scattered observations made by various workers in the course of other studies; these observations will be referred to in the discussions of the experiments conducted by the writer.

MATERIALS AND METHODS.—With the exception of plasmodia of *Physarum polycephalum* Schw., which were derived from sclerotia, plasmodia of all species cultured in the laboratory were obtained from spore cultures. Since there is not enough surface moisture on agar slants to insure good spore germination, cultures were made by adding 1 cc. of sterile distilled water to each slant; spores were then sown directly in the water. Such cultures were placed in the dark, as various workers have shown that light destroys swarm cells. When plasmodia developed, they were maintained in culture by means of plasmodial transplants to fresh nutrient media. Three culture methods were used: (1) the moist chamber method developed by Camp (fig. 7), (2) Petri plate culture (fig. 13), and (3) beaker culture (fig. 9). With *P. polycephalum*, all three methods were employed, whereas only Petri dish cultures were made for *P. tenerum* Rex, *P. compressum* Alb. & Schw., *Didymium xanthopus* (Ditm.), Fr., *Hemitrichia vesparium* (Batsch) Maehr, *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

In moist chamber cultures of *P. polycephalum*, the plasmodia were fed every twenty-four hours by sprinkling ground-up rolled oats directly on them; in both beaker and Petri dish cultures of this same species, three per cent rolled oat agar was used. *P. tenerum*, *P. compressum*, *F. septica*, and *H. vesparium* were grown on two and five-tenths per cent corn decoction agar, adjusted to pH 5.4–5.6; *D. xanthopus* was grown on five per cent carrot decoction agar; *L. fragilis* was grown on both corn decoction agar and three per cent hay infusion, one per cent dextrose agar.

In experiments involving light intensity, the cultures were placed under light bulbs of various wattage, the bulbs being suspended three feet above the open top boxes in which the cultures were placed. All experiments involving such artificial light were conducted in a room in which the temperature varied from 23° to 26°C. Experiments involving direct sunlight were conducted in a greenhouse where the temperature remained at 21°C. in the daytime, dropping to as low as 15°C. at night.

Species herein considered are divided into three groups, based on plasmodial color: yellow pigmented, non-pigmented, and variable plasmodial types, which ranged from white to cream-colored and various light shades of yellow.

YELLOW-PIGMENTED PLASMODIAL TYPES.—This group includes the species whose plasmodia are normally yellow at all times, except for the period immediately previous to the formation of fruiting bodies. In this group are included *Physarum polycephalum* Schw., *P. tenerum* Rex, *Fuligo septica* (L.) Weber, and *Leocarpus fragilis* (Dicks.) Rost.

Physarum polycephalum Schw.—It was possible to conduct many more experiments with this species than with any of the others, because of the ease with which it can be cultured, the rapidity of its growth, and the subsequent formation of relatively large

amounts of protoplasm. As has been previously mentioned, all three types of culture methods were utilized, but the majority of experiments was conducted with moist chamber cultures which were fed equal amounts (0.5 gm.) of pulverized rolled oats every twenty-four hours. It was soon discovered that the plasmodia could not be kept in direct sunlight, although whether the destruction was due to the intensity or quality of the light is not yet known; possibly both factors were concerned.

Preliminary experiments revealed that, except in a few cases, plasmodial cultures of the beaker or Petri dish type would not form fruiting bodies in the complete absence of light if they were inoculated from stock cultures that had been maintained in the dark. This was also found to be true for moist chamber cultures that were fed every twenty-four hours. Several hundred cultures of this type were set up in the dark at various times and only three or four fruited, while check cultures grown in artificial or diffuse natural light nearly always formed sporangia. Those few cultures that did fruit in the dark passed a considerable time (15 days or over) in the vegetative stage. In cultures inoculated from stock plasmodia maintained in strong natural light, a delayed effect was frequently shown, for cultures of this type often fruited in the complete absence of light. Sixty cultures were inoculated from stock plasmodia which had been kept in the light; of these, seventeen (28 per cent) produced sporangia in the dark. In order to overcome any delayed effect, all subsequent experiments were conducted with cultures inoculated from stock plasmodia maintained in the dark. For the sake of uniformity, five-day old stock cultures were arbitrarily chosen as sources of inocula.

Plasmodia grown in light were always a paler yellow than those kept in the dark. Hofmeister (1867, p. 21) stated that plasmodia of *Fuligo septica* (L.) Weber which were kept in the dark were paler than those grown in the light; however, Baranetzki (1876), working with the same species, stated that the reverse was true. The observations of the writer on yellow-pigmented plasmodia tend to confirm the findings of Baranetzki rather than those of Hofmeister. It was also noted in *P. polyccephalum* that plasmodia grown in the dark showed a tendency to spread, whereas the protoplasm of plasmodia grown in light showed a decided tendency to form clumps and large, swollen plasmodial "veins." These two types of plasmodial configurations are shown in figures 4 and 5.

The finding that light was nearly always essential for the fruiting of this species, under the conditions outlined, naturally suggested that the intensity of light might condition the time of fructification, and it was subsequently found that a definite correlation exists between light intensity and duration of vegetative phase. Over a period of two years moist chamber cultures were grown in open top boxes under 60, 100, 150, 200, and 400 (two 200's) watt bulbs, suspended three feet above the cultures, and

it was found that under each type of light (with the exception of the most intense) a rather definite fruiting rhythm was exhibited. The following list summarizes these data:

Cultures under 60 watt light fruited in 12 days					
"	"	100	"	"	11
"	"	150	"	"	10
"	"	200	"	"	9
"	"	400	"	"	6-9

There were some variations from the above figures, but, in the great majority of cases, the fruiting occurred with such regularity that the day of sporangium formation of a given plasmodial culture could be correctly predicted on the day it was started. The analyses of lights from the various types of bulbs used in the experiments show that variations in the length of time spent in the vegetative phase were due to the factor of intensity rather than to differences in quality of the various lights. Analyses of the types of bulbs used are shown in table 1.²

TABLE 1. Analyses of electric light bulbs used in light intensity studies.

Lamp size (watts)	Approximate lumens	Percentage of total light			
		Violet and blue	Green	Yellow	Orange and red
60	760	2.25	40.25	27.5	30.0
100	1530	2.3	40.7	27.4	29.6
150	2535	1.9	41.0	27.0	30.1
200	3400	2.5	41.5	27.2	28.8

In discussing various features of the plasmodial behavior of *Didymium difforme* (Pers.) Duby, Skupienski (1928, p. 329) made the statement: "... if one submits a well-expanded plasmodium to the action of light rays, coming from a well-defined source, for two minutes only, and if one repeats this short illumination every two hours, the fruiting of the plasmodium in question is surely accelerated." This suggested the possibility that fruiting might be induced more rapidly by alternate periods of light and darkness rather than by continuous exposure. Moist chamber cultures were exposed to light from a 100 watt bulb for eight out of each twenty-four hours and were found to require one to two days longer for fruiting than those cultures continuously exposed to the same light. Cultures which were exposed to intermittent illumination from a 60-watt bulb (obtained by means of a flashing device that gave periods of 2.5 seconds of light alternating with periods of 0.5 second of darkness), remained in the vegetative state two days longer than cultures exposed to continuous illumination from the same type of bulb. It would seem from these results that alternate periods of light and darkness enable plasmodia to fruit with a much smaller total amount of light than required by plas-

² From the manufacturer's published tables.

media when they are continuously exposed. The cultures exposed to light from a 100-watt bulb for eight hours per day required only one or two days longer for fruiting than cultures continuously exposed, whereas, if a direct mathematical relationship existed, theoretically they would require thirty-three days, since they were exposed to light only one-third of the time. What actually occurred was that cultures continuously exposed required a total of 264 hours exposure to light for fruiting, while cultures exposed eight hours per day required only 96 to 104 hours of exposure, although they did spend a longer time in the vegetative phase than cultures continuously exposed.

TABLE 2. *Light transmission analyses of colored plates.*

Plate used	Wave length (millimicrons)						
	400	450	500	550	600	650	700
Blue	68.00	45.45	6.46	2.56	0.57	0.52	24.46
Red	6.00	0.56	3.29	3.84	16.09	33.68	43.61
Yellow ..	4.00	2.27	10.52	21.79	33.33	25.78	39.26

Experiments relative to the effect of lights of various wave lengths (of the visible spectrum) were first conducted with moist chamber cultures which were continuously exposed to lights transmitted through colored glass plates. One series was conducted, and then standardized gelatin filters were substituted for the glass plates. In the original experiment three colored plates were used: red, blue, and yellow. These plates were analyzed by means of a Raze-Mulder Color Analyzer; results of the light transmission analyses are summarized in table 2. From this table it may be seen that the blue plate passed a comparatively high percentage of light in the violet and blue bands of the spectrum, very little in the green and yellow, practically none in the orange, but

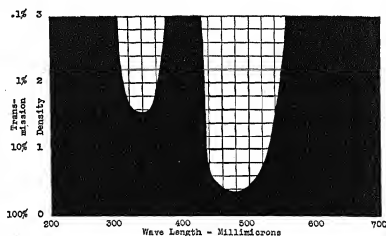
TABLE 3. *Summary of results of one experiment using light transmitted through variously colored plates.*

Culture number	Color of plate used	Hours per day exposed	Length of vegetative phase
1	Blue	24	15 days
2	"	8	14 "
3	Red	24	Died
4	"	8	16 days
5	Yellow	24	Died
6	"	8	14 days

passed considerable light in the red and near infra red. The red plate passed very little in the violet and blue bands, from 3.9 to 16 per cent in the green and yellow, increasing in the orange and red. The light transmission of the yellow plate was roughly similar to that of the red plate; however, it trans-

mitted a greater percentage of light in the green, yellow, and orange bands.

Six cultures were used in the preliminary experiment, the results of which are given in table 3. This experiment showed that the shorter wave lengths of the visible spectrum stimulate fruiting, while the longer wave lengths either retard or prevent fruiting. In later experiments in which Wratten Filters were used, only one, whose transmission analysis is shown in figure 1, induced fruiting. This would tend to support the view that the shorter wave lengths are necessary for fruiting. On the other hand, in the longer wave lengths of the visible spectrum or in infra-red rays, the plasmodia never fruited under conditions of continuous exposure.

Fig. 1. *Light transmission analysis of gelatin filter under which fed cultures of P. polycephalum formed fruiting bodies.*

A number of investigators have maintained that the fruiting of myxomycetes is due solely to the depletion of nutrition. Camp (1937) has shown that plasmodia of *P. polycephalum* may be induced to fruit by starvation. The present study indicates that cultures which are not fed will spend less time in the vegetative phase than those which are fed, but this will scarcely explain why plasmodia will

TABLE 4. *The effect of light intensity on fruiting in unfed cultures of Physarum polycephalum.*

Lamp (watts)	Number of cultures	Average length of time required for fruiting
60 W.	8	87 hours
100 W.	6	81 "
150 W.	21	78 "
400 W.	21	68 "

often fruit when they are supplied with abundant food materials. In order to determine whether or not light would have any effect on plasmodia to which no nutrient was supplied, a number of cultures were set up. As in previous experiments, five-day old stock cultures, which had been fed twenty-four hours previously, were used as sources of inocula. With few exceptions the light reactions were the same as those

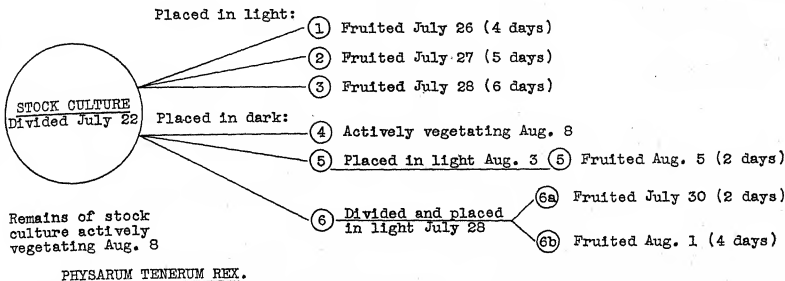


Fig. 2. Diagrammatic representation of the effect of diffuse natural light upon the fruiting of *P. tenerum*.

shown by fed cultures. A higher percentage of unfed cultures (inoculated from stock plasmodia maintained in the dark) formed sporangia in the complete absence of light than did fed cultures of the same type. Results of light intensity studies conducted with cultures of this type are summarized in table 4.

Experiments concerned with exposing unfed cultures to various wave lengths of light did not produce results which were in agreement with the results obtained when fed cultures were used. Sporadic fruiting occurred in such cultures, regardless of the light used, so the inference is that the influence of starvation is much stronger than any possible inhibitory effect of light in the longer wave bands.

Physarum tenerum Rex.—This species has the same type of bright yellow plasmodium as *P. polycephalum*, but as yet has not been induced to form large masses of protoplasm. Like *P. polycephalum*, plasmodia of *P. tenerum* grown in the light were a paler shade of yellow than those grown in the dark. A clumping of plasmodia occurred in the light but to a lesser degree than in *P. polycephalum*.

Numerous plasmodial cultures were exposed to direct sunlight in the greenhouse, and after forty-eight hours all had lost their color. When these cultures were placed in the dark, they failed to revive, thus showing that plasmodia of *P. tenerum*, like those of *P. polycephalum*, are killed by direct sunlight. In no case did plasmodial cultures fruit in the complete absence of light; over two hundred cultures were kept in the dark, and all remained in the active vegetative state as long as the nutrient agar remained moist. Many plasmodia remained active for as long as sixty days, and one persisted for seventy-six. Due to the fact that it needs transplanting so infrequently, this species is excellently adapted for laboratory cultivation, although, as mentioned above, it does not produce such large quantities of protoplasm as *P. polycephalum*. Preliminary experiments indicate that it can probably be adapted to moist chamber culture, using pulverized rolled oats as nutrient.

In diffuse natural light in the laboratory, different lengths of time were required for the formation of fruiting bodies by various plasmodial cultures. The results of one such experiment are shown in figure 2. This figure shows that the vegetative phase of *P. tenerum* varied from 2 to 6 days in natural light and well illustrates the necessity for more controlled conditions than are normally found in the laboratory. Seifriz and Russell (1936) apparently encountered similar difficulties, since they found that under laboratory conditions the rhythm of *P. polycephalum* varied from 9 to 23 days—a situation non-existent for this species (*polycephalum*) under conditions of constant light, temperature, and food supply. Under such controlled conditions some variation did occur in cultures of *P. tenerum*, but this variation was not nearly so great as that exhibited by cultures grown in natural light. The average length of the vegetative phase of twenty-four cultures grown under a 100-watt light was eighteen days, while under a 400-watt light the average length of the vegetative phase was thirteen days (15 cultures used). This difference is sufficient to show that the same relation exists between length of vegetative phase and light intensity as was exhibited by *P. polycephalum*. Plasmodia of *P. tenerum* possess a vegetative phase of somewhat longer duration than plasmodia of *P. polycephalum*.

Fuligo septica (L.) Weber.—Spores of this species germinate readily, and small, yellow plasmodia have frequently been obtained from spore sowings on corn decoction agar. These plasmodia, which failed to attain the size of those found in nature, grew very slowly, and none was ever observed to complete its life cycle. Attempts to maintain this species in moist chamber culture for any length of time have also failed; hence, all observations on this species have necessarily been made on plasmodia collected in the field. From these observations alone, it would seem that *F. septica* must have light in order to complete its life cycle, because young plasmodia, which were collected in more or less shaded habitats and kept in darkness, have never been observed to form fruiting bodies.

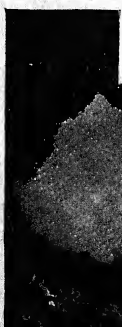
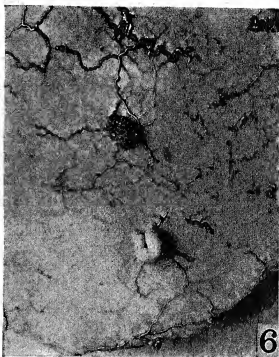
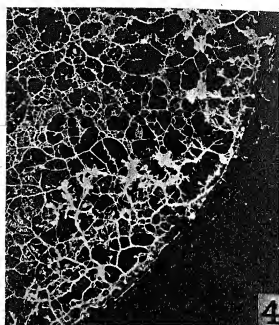
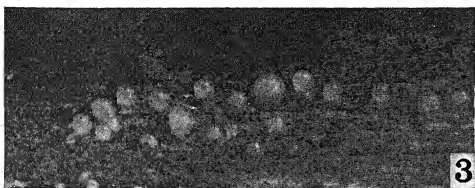


Fig. 3-9.—Fig. 3. Immature sporangia of *H. clavata*. $\times 6$.—Fig. 4. Portion of plasmodium of *P. polycephalum*, grown in darkness. Natural size.—Fig. 5. Portion of plasmodium of *P. polycephalum*, grown in light. Natural size.—Fig. 6. Plasmodium of *P. septica*, showing clumping of protoplasm. $\times 1\frac{1}{2}$.—Fig. 7. Moist chamber culture of *P. polycephalum*. $\times \frac{1}{3}$.—Fig. 8. Immature sporangia of *T. persimilis*. $\times 2$.—Fig. 9. Beaker culture of *P. polycephalum*. $\times \frac{1}{4}$.

Plasmodia of *F. septica* show the phenomenon of clumping in a remarkable degree when exposed to light. For this study, pieces of wood, on which active plasmodia were spread, were placed in moist chamber culture, and in a few hours most of the plasmodia had moved to the moist paper on which the pieces of wood were placed. When such plasmodia were exposed to light from a 100-watt bulb, at a distance of eighteen inches, a noticeable congregation of protoplasm into the larger veins of the plasmodial network became apparent at the end of one minute. At various points along these veins, swellings began to appear, and four to five minutes later some of them had

spore sowings on either corn decoction agar or hay infusion agar. These plasmodia need transplanting infrequently (every 2 or 3 weeks) and hence are easily maintained. No fruiting has been obtained as yet, and the suggestion is made that neither of the two media used is adapted for its cultivation. Like the yellow-pigmented types previously discussed, the plasmodia of *L. fragilis* clump and are bleached when exposed to light and are killed when exposed to direct sunlight.

NON-PIGMENTED PLASMODIAL TYPES.—In this group are placed those species in which the plasmodia are typically watery- or pearly-white. All species in the

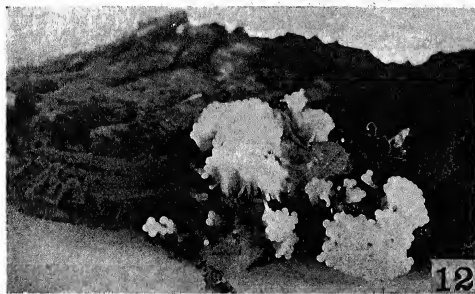
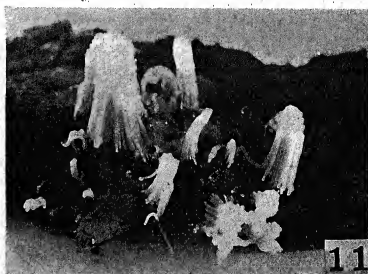


Fig. 10-13.—Fig. 10. Plasmodium of *S. fusca*. $\times 3$.—Fig. 11. Same as fig. 10—6½ hours later. $\times 3$.—Fig. 12. Same as fig. 10—4½ hours later. $\times 3$.—Fig. 13. Petri dish culture of *D. xanthopus* on carrot agar. Natural size.

grown as large as 1 cm. in diameter (fig. 6). Baranetzki (1876) mentioned the clumping of plasmodia of *F. septica* exposed to light but failed to mention the production of large plasmodial swellings such as those just described. Plasmodial cultures of *F. septica* on corn decoction agar were killed within twenty-four hours when exposed to direct sunlight.

Leocarpus fragilis (Dicks.) Rost.—In so far as a source of small plasmodia is concerned, this species is an excellent yellow-pigmented type for laboratory cultivation. Spores germinate readily, and plasmodia can generally be obtained in ten days or less from

group herein discussed change color before the fruiting bodies mature, but the change occurs after sporangial delimitation has begun.

Physarum compressum Alb. & Schw.—The pearly-white plasmodium of this species was obtained easily from spore sowings on corn decoction agar. Plasmodia formed typical sporangia in the absence of light about six days after they were large enough to be visible without the aid of a lens. Attempts to maintain the species in culture by means of plasmodial transplants were, on the whole, unsuccessful. Disturbing the plasmodia markedly inhibits growth

and fruiting, and cultures made by means of plasmodial transplants nearly always died within twenty-four hours, although in a few cases the life cycle was completed. Either the plasmodium of *P. compressum* is exceptionally delicate or else corn decoction agar is not the most suitable medium for its cultivation. However, attempts to grow this species on various other types of media yielded no better results.

Physarum nutans Pers.—Only one observation was made on the light relationships of this species. A small, white, net-like plasmodium, found under the bark of a decaying log of *Prunus serotina*, was transported to the laboratory, kept in complete darkness, and, after three days, formed typical sporangia of *P. nutans*. The plasmodium received no light except for a few moments when it was first discovered, so it is safe to infer that light is not prerequisite for the completion of its life cycle. Since the age of the plasmodium was unknown, no definite length of vegetative phase may be set.

Stemonitis spp.—The plasmodia of approximately half of the described species in the genus *Stemonitis* have not yet been observed, but in the majority of known cases they are white. This is true for both *Stemonitis axifera* (Bull.) Macbr. and *S. fusca* Roth (fig. 10, 11, 12), the plasmodial behavior of which has been described previously by the writer (Gray, 1936, 1937). All conclusions concerning these species are based on observations made on plasmodia collected in their natural habitats, as there are no records of either species having been artificially cultured, and to date no methods for their cultivation have been devised. From observations made by the writer, it is evident that light is not necessary for fruiting, since plasmodia which arose on decayed wood in moist chambers were able to complete their life cycles in the absence of light. Several observations have also been made on plasmodia which were collected under the bark of decaying logs and transported in darkness to the laboratory; fruiting of such plasmodia subsequently occurred in the complete absence of light. As in other species, in which the plasmodia inhabit the interstices of decaying wood, it will be difficult to establish a definite length of time for the vegetative phase; however, data at hand indicate that plasmodia of *S. fusca* remain in the vegetative stage somewhat longer than do plasmodia of *S. axifera*.

Comatricha typhoides (Bull.) Rost.—In a recent paper (Gray, 1937) the writer described the development of this species from white plasmodium to mature sporangia. The time required for completion of fruiting in the specimen described was twenty-three hours. Another such observation has been made, and the required time was fifteen hours. This variation in time was probably due to difference in ages of plasmodia, and since plasmodia of this species are similar in habit to those of various species of *Stemonitis*, it will be difficult to determine the normal length of time which they spend in the vegetative stage.

C. typhoides apparently does not need light in order to fruit, since plasmodia have frequently ap-

peared and eventually formed sporangia on moist pieces of decayed wood kept in the dark.

Lamproderma arcyrionema Rost.—Spores of this species may be germinated easily in either tap- or distilled water, but as yet no plasmodial development has been obtained on any of the artificial media used. Plasmodia have been obtained, however, by sowing spores on small pieces of moist, decayed wood kept in the dark. Sporangia have been obtained in this fashion in the complete absence of light, but no data are available concerning the exact length of the vegetative phase. Like the previously described members of the order Stemonitales, *L. arcyrionema* possesses plasmodia which inhabit the interior of decaying wood; hence the age of plasmodia cannot be accurately determined. Since in several instances small, pearly-white plasmodia of this species arose on moist wood and formed sporangia in 12–14 hours, we may infer that *L. arcyrionema* possesses a relatively short rhythm in so far as the vegetative stage is concerned.

Hemitrichia vesparium (Batsch) Macbr.—The plasmodium of this species is listed in most taxonomic works as being deep red or purple red, but the writer has never observed anything but white plasmodia during the active vegetative phase. It is true that the color becomes red before the fruiting bodies are mature, but this color change occurs after sporangial delimitation has begun and not while plasmodia are still vegetating. It is recognized that plasmodial colors may be somewhat variable, depending upon the color of the substratum, but generally a radical change in plasmodial color during the vegetative phase is due simply to the engulfing of different colored particles of the substratum. Naturally, if a plasmodium should engulf red particles it would, for a time, appear red. This type of change in plasmodial color is exemplified by the photograph of a plasmodium of *F. septica* (fig. 6); at the top the color is brownish-yellow due to the engulfing of many, small, brown fragments of decayed wood on which it was growing before it was allowed to spread to the paper in the moist chamber.

H. vesparium needs no light in order to form sporangia. Numerous instances have occurred in which plasmodia have come to the surfaces of pieces of decayed wood, which had been placed in the dark, and there formed sporangia. Sporangia of *H. vesparium* have also been found on the walls and ceilings of mushroom houses, where the only light was an occasional flashlight beam.

Spore sowings in tubes of corn decoction agar have yielded pearly-white plasmodia which grow rapidly and are easily maintained through plasmodial transplants. In this type of culture fruiting was very erratic, so no data concerning rhythm or effect of light intensity are available. Fruiting frequently occurred, but in no case was an entire plasmodium utilized in sporangial formation. In several cultures, one sporangium was produced by a small bit of plasmodium and then was engulfed and destroyed almost immediately by the remaining plasmodial mass. As

yet the factors inducing fruiting in *H. vesparium* in this type of culture are unknown.

Hemitrichia spp.—Except for the species just discussed, no other species of the genus *Hemitrichia* were grown in the laboratory. However, several naturally occurring plasmodia of *H. clavata* (Pers.) Rost. (fig. 3) and *H. stipitata* (Masse) Macbr. were observed to form sporangia in the complete absence of light. Both species have been found in mushroom houses where they had fruited in darkness. The writer has observed only white plasmodia of either species, although Lister (1925, p. 221) states that the plasmodium of *H. clavata* may be either watery-white or rose-red. Sporangia of both species have been observed to pass through a rose-red stage, but this change of color occurs after sporangial delimitation has started.

made on twenty-two plasmodia of this species which fruited in the dark.

VARIABLE PLASMODIAL TYPES.—One species, *Didymium xanthopus* (Ditm.) Fr., has been placed in this separate group because of the variability of its plasmodial color. In the discussions of various yellow-pigmented plasmodial types it was noted that the bright yellow color fades to a certain extent when plasmodia are placed in the light. Plasmodia of *D. xanthopus* did not exhibit this type of variability, as any given plasmodium might vary from white, through various shades of creamy- or greenish-yellow to bright yellow, independently of light conditions. For instance, two plasmodial transplants might be made from a single yellow (or white) plasmodium, and the plasmodium of one of the resultant cultures would be white or cream-colored, whereas the other would

TABLE 5. Summation of observations on twenty-two plasmodia of *T. persimilis* which completed their life cycles in complete absence of light.

No. of plasmodia	Source of plasmodia	Time of collection	Fruiting completed	Hours required
1	Under bark of decaying cottonwood log	2:00 P.M. Sept. 8	8:00 A.M. Sept. 9	18
3	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:00 A.M. Sept. 14	17
1	Under bark of decaying wild cherry log	3:00 P.M. Sept. 13	8:00 A.M. Sept. 15	31
3	Appeared on moist wood in dark chamber	10:00 A.M. Sept. 14	8:00 A.M. Sept. 15	22
7	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	11:00 P.M. Sept. 15	9.5
3	Under bark of decaying wild cherry log	1:30 P.M. Sept. 15	8:00 A.M. Sept. 16	18.5
4	Under bark of decaying wild cherry log	2:00 P.M. Sept. 17	8:00 A.M. Sept. 18	18

Trichia persimilis Karst.—The plasmodium of this species is typically watery-white and does not change color until the separate sporangia have taken form (fig. 8). After the sporangia have assumed the shape and size of mature fruiting bodies, they become pale lemon-yellow and gradually darken until the shining golden- or yellow-ochraceous color of mature sporangia is attained. Attempts to culture this species in the laboratory on various types of media used for other species have failed, but numerous observations have been made on plasmodia collected in the field and brought to the laboratory. These plasmodia were all collected beneath the bark of decaying logs, where they received no light; they were then transported in darkness to the laboratory where most of them were placed in moist chambers in the dark. Fruiting bodies were formed both by sporangia kept in the dark and those kept in diffuse natural light. Since the age of each plasmodium is unknown, no definite rhythm may be set for *T. persimilis*; the indications are, however, that it has a comparatively short rhythm. Table 5 is a summation of the observations

be yellow. Plasmodia, regardless of color, formed sporangia equally well under conditions of artificial light or darkness, although there was no regularity in the duration of the vegetative phase of the various cultures—fruiting occurring any time between six and twenty-seven days. Plasmodial cultures were maintained in direct sunlight for periods as long as twenty days, but no cultures ever formed sporangia under such conditions.

It is evident from these results that light plays no important role in sporangia formation, and at present no theory is presented as an explanation of what causes fruiting in this species. There is a possibility that temperature is an important factor, since Skupienski (1934) maintains that different strains of this species have different optimum temperatures, although the race which he terms "*thermophilum*" (not implied in a systematic sense) showed very little variation in length of vegetative phase under temperature conditions varying from 6° to 25°C. If physiologic strains do exist, the interpretation of the results of other investigations, as well as the present

one, becomes a difficult task, since different strains may be involved in the various studies.

DISCUSSION.—From the above observations, it is evident that under the conditions outlined yellow-pigmented plasmodia will not form fruiting bodies in the complete absence of light. This leads immediately to the conclusion that the pigment has an important function and is probably a part of the mechanism by which plasmodia are able to perceive the stimulus of light. The fundamental role of pigments in various living processes has been shown with many organisms, plant and animal, so this finding is not that of a new phenomenon but merely another example of a long-known one.

The chemical nature of the pigment has not been determined, but several theories have been advanced as to the nature of pigments that occur in myxomycetes. Seifriz and Zetzmann (1935) suggested that the yellow pigment of *Physarum polycephalum* belongs to the lyochrome or flavone group (of which the flavins are members). If the pigment is of the flavone type, a plant relationship would be suggested for the group, since such pigments as a rule do not occur in animal tissues. Solacolu (1932), working with pigments from the mature fruiting bodies of twenty-six species of myxomycetes, came to the conclusion that pigments occurring in these organisms are anthracenes. He also suggested a plant relationship, since anthracenes are found in some fungi and some higher plants, but generally not in animals, although phenanthrene, an isomer of anthracene, and its derivatives are known to occur in animals (Fieser, 1936). Whatever the chemical nature of these pigments may be, it is evident that light has a specific photochemical effect upon them, since plasmodia were observed to become paler when exposed to light. Stobbe (1908), working with various fulgides, has shown that these complex chemical substances not only change color when exposed to light but also that lights of various wave lengths cause different changes. For example, this worker found that triphenylfulgid became orange-yellow when exposed to light of 550 to 700 millimicrons but became dark brown at wave lengths of 440 to 550 millimicrons. The fact, as shown by Stobbe, that light of various wave lengths may induce specific photochemical effects may explain in part the behavior of plasmodia of *P. polycephalum* when exposed to the different wave bands. The work of Brandza (1926) would indicate that profound morphological changes may occur in myxomycetes as a result of the influence of light. This worker reports that plasmodia of some species can live in direct sunlight and that such plasmodia are extremely modified. For example, he states that *Physarum viride* (Bull.) Pers. and its variety *incanum* Lister are really forms of *P. nutans* Pers., which were derived from different colored plasmodia by exposure to direct sunlight. Such change in color indicates a definite photochemical effect.

It was noted that yellow-pigmented forms showed a tendency to clump when exposed to light, a finding

in agreement with the results reported by other investigators. Baranetzki (1876), working with *Fuligo septica*, first mentioned clumping of protoplasm of plasmodia; this worker further stated that a slight increase in illumination causes a distinct retardation of streaming movements of plasmodia. Englemann (1879), working with the amoeba, *Pelomyxa palustris*, noted that when light is thrown upon a pseudopod of this organism, it is withdrawn suddenly. Mast (1911, p. 229) makes the general statement that light retards the activity of protoplasm and prevents the formation of pseudopods on the more highly illuminated side. Thus we can see that light causes a contraction of protoplasm of organisms other than myxomycetes, and, while it may simply be an orientation process with respect to the stimulus of light, it would appear to be more than a coincidence that plasmodia not only clump when exposed to light but also when about to form fruiting bodies, as Baranetzki has already pointed out.

Various workers (Baranetzki, 1876; Stahl, 1884, p. 167; Davenport, 1879) have shown that plasmodia of myxomycetes, which are known to react to light, are negatively phototropic. The anomaly presented by a negatively phototropic organism requiring light for one of its most important processes is evident. The fact that the above workers have demonstrated this negative phototropism does not necessarily mean that plasmodia will always react negatively, however, since it is known that certain organisms may change their tropic responses. For example, Keeble (1910, p. 41-42), working with two species of *Convolvata*, found that in bright light *C. roscoffensis* is positively and *C. paradoxa* negatively phototropic, while in dim light, *C. roscoffensis* is non-phototropic and *C. paradoxa* is positively phototropic. Loeb (1904) has shown that certain fresh-water crustaceans (*Gammarus*, *Cyclops*, *Daphnia*), which are naturally negatively phototropic, can be induced to reverse their tropic responses through the use of certain chemicals, particularly acids. It may well be that myxomycetes, like other organisms, have the power of changing their phototropic responses.

The fact that under controlled conditions of light, temperature, and nutrition, plasmodia fruited with great regularity would support the earlier view of Cayley (1929) that a certain periodicity (rhythm) exists in the production of spores. Among the Fucaceae, the periodical liberation of sexual cells has been described by several workers. Williams (1898, 1905), who noted a fortnightly liberation of sexual cells of *Dictyota dichotoma* off the coast of England and Wales, concluded that this periodicity was hereditary, but that in seas where there are tides, it might be influenced by the changes of amount of illumination. Hovt (1907) described a monthly liberation of sexual cells of this same species at Beaufort, North Carolina, and Lewis (1910) found that it produced fortnightly crops at Naples and noted that the critical points in the life cycle (initiation of sexual cell rudiments and liberation) not only bore a relationship to the tides,

as shown by previous workers, but coincided exactly with the periods of maximum intensity of illumination. This latter worker hypothesized that *Dictyota*, in adapting itself to differing conditions at various localities, has acquired its habit of periodicity in response to different factors. Tahara (1909) described an identical periodicity in the fortnightly liberation of oögonia of *Sargassum enerve*; in a later work (1913), Tahara showed that the initial liberation in *S. enerve*, *S. Horneri*, and *Cystophyllum sisymbrioides* bore a relationship to the highest spring tide, but that the intervals between successive liberations varied, thus suggesting the possible influence of other environmental factors (illumination?). Keeble (1910, p. 33) found that light was a very important factor in the egg-laying of *Convoluta roscoffensis*. He found that alternation of six hours of exposure to light with eighteen hours of darkness was the optimum for egg-laying and noted that this was the same exposure received by *C. roscoffensis* during spring tidal periods at which its eggs are laid habitually.

In relation to the rhythmical fruiting of myxomycetes, Williams' view that periodicity might be hereditary is supported in part by the fact that certain species exhibit longer vegetative phases than others. For example, *Physarum tenerum*, under a 100-watt light, required eighteen days for fruiting, whereas, *P. polycephalum*, under identical conditions, required only eleven days. While rhythmical fruiting may possibly be hereditary to a certain extent, it is certainly not so innate as to be unalterable with respect to environmental factors. The writer has shown that in fed cultures of *P. polycephalum* fruiting rhythm may be changed by varying the intensity of illumination. Camp (1937) showed that by starvation of plasmodia the vegetative phase of this species could be markedly reduced. Experiments of the writer, involving both starvation and intensity of illumination, showed that both factors inter-reacted in influencing the time of fruiting. Differences in rhythms of species, then, tend to support the view

that rhythmical fruiting is hereditary; yet experiments on yellow-pigmented plasmodia, involving lights of different intensities, show that this periodicity of fruiting may be modified.

SUMMARY

Our lack of knowledge of the biology of the myxomycetes is due to the fact that so few species have been cultured. The problem of immediate interest is the development of methods for culturing many species under laboratory conditions.

The effects of light upon the fruiting rhythms of four yellow-plasmodial types, ten non-pigmented plasmodial types, and one variable plasmodial type (*Didymium zanthopus*) were investigated. It was found that, with the species studied, yellow-pigmented types require light in order to complete their life cycles, while non-pigmented types and *D. zanthopus* fruit equally well in light or darkness.

Under controlled conditions of temperature, light, and nutrient, fruiting periods of pigmented types assumed great regularity. Under conditions of constant temperature and continuous illumination the lengths of the vegetative phase of *Physarum polycephalum* and *P. tenerum* are conditioned by the total amount of light received. Under conditions of intermittent illumination the vegetative phase of *P. polycephalum* may be lengthened, although the total amount of light necessary for a cycle to be completed may be greatly reduced. Fed cultures of *P. polycephalum*, when placed in lights of various wave lengths, formed sporangia only when exposed to the shorter wave lengths of the visible spectrum. The plasmodia of all yellow-pigmented types clumped and faded when they were exposed to light.

The laboratory cultivation of *Physarum compressum*, *P. tenerum*, and *Hemitrichia vesparium* is reported for the first time.

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A METHOD FOR THE CULTURE OF EXCISED PLANT PARTS¹

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THE CULTURE of excised plant organs as a method of approach to problems of intermediary metabolism has been seriously restricted in its usefulness by the necessity for maintaining sterile conditions in the culture medium. Klein and Kisser (1924) have published a monograph on the sterile culture of higher plants in which they point out many of the difficulties to be encountered in the sterilization of plant parts by chemical means. Kultzscher (1932) and Klein and Linser (1933), however, report successful results obtained by the sterilization of plant stems with dilute bromine water followed by aseptic transfer of the sterilized leafy shoots to flasks containing autoclaved culture solutions. Attempts to duplicate the techniques of these investigators on a large scale in this laboratory have failed to yield satisfactory results. Therefore, physiological means of controlling the activity of microorganisms in the culture solutions were investigated. A low temperature method was developed which gave excellent results in repeated trials and which permitted the ready handling of large numbers of plants. A description of this method as applied to large leafy shoots of tobacco plants follows.

Each plant was carefully removed from its container, and the soil or sand was removed from the roots with running water. The lower end of the stem was then submerged in flowing water, while the roots were removed by a clean slanting cut in the transition zone between root and stem tissues. The cut stem was immediately transferred to a flask contain-

ing the desired solution. The cultures were either placed in gallon crocks to which cracked ice was continually added or immersed in cold running water in large stone sinks.

The low temperatures (5°C. to 10°C.) to which the solutions were subjected prevented visible growth of microorganisms during the experimental period without materially interfering with the absorption of the culture solutions. Two comparable groups of Havana Seed tobacco plants, when cultured in solutions maintained at temperatures of about 5°C. and 20°C., respectively, absorbed almost identical volumes of solution. That the absorption of water by cut stems is not dependent upon physiological processes in the stem tissues was shown by killing the lower portions of the stems with strong acid or with solutions containing ammonium salts. Stems so injured were still able to absorb and conduct the culture solutions to the leaves without apparent diminution in rate. In general, tobacco shoots bearing from ten to fifteen leaves each may absorb from 200 to 500 ml. of the chilled solution in four days, depending on the nature of the solute present. In repeated instances, glutamic acid and its hydrochloride, nicotine hydrochloride at pH 3.4, and nicotinic acid hydrochloride increased both the rate of absorption and the total quantity of solution absorbed per plant. It is interesting to note that the monopotassium salt of glutamic acid exerted no such effects.

It seems probable that contamination of the culture solutions might be more efficiently controlled by the use of insulated metal tanks designed to receive culture flasks of the desired size and containing water regulated by a refrigerating unit to a temperature of about 5°C.

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Contribution from the Osborn Botanical Laboratory, Yale University.

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Several external factors appear to influence the success of cultures of this type. It has been observed that an acidity of pH 3.0 or somewhat lower not only facilitates the absorption of the culture solution but also restrains the growth of bacteria. A low relative humidity, a high atmospheric temperature, and exposure to direct light during the first twelve hours of culture greatly increase the total amount of solution absorbed per plant. After the first day, however, the rate of absorption slows down, and it is usually necessary to place the plants in diffuse light or in total darkness to prevent permanent wilting. This is not the case where excised leaves are employed, particularly if the leaves are not fully developed. Some varieties of tobacco appear to be less adapted to cut stem culture procedures than are others. By the use of plants grown in the greenhouse, it has been observed that Connecticut Broadleaf No. 38 plants may remain turgid and active much longer than plants of the Havana Seed and Rosenberg varieties when exposed to the light. In the dark, the plants of all varieties tested appear to be equally usable. In general, plants grown during the spring and summer months take up larger quantities of solution and remain turgid for longer periods of time than do plants grown during the autumn and winter months.

Evidence was desired to show whether or not the cut stems were able to take up the solute as well as the aqueous solvent during cut stem culture. In two test experiments employing 0.014 molar solutions of nicotinic acid hydrochloride, excised tobacco plants were permitted to take up the entire volume of culture solution which had been placed at their disposal. In neither case was an appreciable residue left behind in the empty flasks. A third experiment was conducted to determine whether or not the solute was transported to the leaf blades. Six Connecticut Broadleaf No. 38 tobacco plants were placed as described above in flasks containing 0.007 molar solutions of nicotine (Eastman, BP 115–117°C. at 12 mm.) adjusted to pH 3.4 with hydrochloric acid. A similar number of plants was placed in tap water adjusted to pH 3.4, and a third group was placed in tap water at pH 8.0. After four days of culture at a solution temperature of 10°C., the plants were harvested and dried in a current of air preheated to 80°C. Analyses for nicotine indicated no difference in the total nicotine present in the leaves of the two groups of plants in water. The plants supplied with nicotine, however, had taken up and conducted 1.58 gm. of nicotine to their leaves. Nicotine to the extent of 0.34 gm. remained in the culture solutions. The remainder, 1.50 gm. of the alkaloid, might easily

have been located in the stems, since the dry weight of stem tissues per plant generally exceeded that of the leaf blades. Such evidence leaves little question as to the ability of the cut stems to take up and conduct the solutes to the leaves.

As yet it is possible to maintain the activity of leaves or leafy shoots of tobacco plants for only limited periods of time when they are separated from the plant roots. The metabolism of excised tobacco leaves during culture in light and in darkness has been very thoroughly investigated by Vickery et al. (1933, 1937). It is apparent from this work that fundamental changes of a catabolic nature occur in the leaves soon after removal from the plant. The same changes probably occur in the leaves of excised shoots. In the present study excised shoots in the light remained apparently normal in appearance and gained in dry weight for periods of four to five days. Beyond that time permanent wilting and visible chlorophyll decomposition usually occurred. Attempts to prolong the culture period by the use of either glutamic acid or nicotinic acid hydrochloride in the culture solutions have so far given encouraging results. Supplying these substances to the plants through the cut stems has invariably resulted in increasing the rates of solution uptake and of dry weight accumulation. In addition, permanent wilting and chlorophyll decomposition were substantially delayed (Dawson, 1938). Routine use in the culture solutions of these and of substances possessing similar effects, however, must be delayed until their influence upon the chemical composition of the leaves is more clearly understood.

SUMMARY

A method has been developed by which the excised leafy shoots of tobacco plants were cultured in solutions containing organic substances. Decomposition of the solutes by microorganisms was prevented for all practical purposes by chilling the culture solutions to between 5°C. and 10°C. The undesirable effects of chemical sterilization upon plant tissues and of high temperatures upon the composition of the nutrient solutions were thus avoided. The low temperatures employed did not materially affect the ability of the cut stems to absorb the culture solutions.

Several external factors are discussed which appear to influence the success of cut stem and excised leaf cultures.

Evidence is presented to show that the solutes are readily taken up by the cut stems and conducted in appreciable quantities to the leaves.

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NOTES ON ZYGNEMATACEAE¹

Edgar Nelson Transeau

FOLLOWING ARE descriptions of thirteen new species belonging to the genera *Zygnema*, *Mougeotia*, and *Spirogyra*, that have accumulated in my collection. There is also a new figure (No. 4) for *Zygnema pawhuskiae* Taft, which was described in 1934 and erroneously illustrated by a figure (No. 61) originally drawn for *Zygnema carinatum* Taft, but which had been discarded because punctations on the spore wall were too small.

Zygnema crassiusculum sp. nov.—Vegetative cells 52-58 μ \times 52-144 μ , conjugation scalariform, gametangia cylindric or enlarged; zygospores, formed in one of the gametangia, compressed globose to ovoid, (50-)54-62(-68) μ \times 45-55 μ ; outer wall transparent; median wall of two layers, of which the outer is more or less keeled, scrobiculate, with pits 2-3 μ in diameter and about the same distance apart; the inner median wall is finely and irregularly verrucose.

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Papers from the Department of Botany, The Ohio State University, No. 399.

Cellulis vegetativis 48-58 μ \times 54-100 μ , conjugatione scalari; gametangia recipientibus cylindricis vel paululum inflatis; zygosporis globosis vel subglobosis in gametangio ortis diam. 61-68 μ , 64-71 μ crassis; episporio hyalino; mesosporio bruneo duplici, membrana exteriore scrobiculata, scrobiculis diam. circa 2 μ , eodemque intervallo dispositis, membrana interiore irregulariter verruculosa.

South Africa: Cape Town Flats. Coll. Miss Edith L. Stephens No. 926. (Fig. 3, 3a.)

Mougeotia Drouetii sp. nov.—Vegetative cells 15-18 μ \times 90-180 μ , chromatophore with 4 to 8 pyrenoids in a single row; conjugation scalariform, zygospores compressed spherical or ovoid, 32-36 μ \times 28-34 μ , extending into or across both gametangia, with a highly granular brownish residual membrane lining the gametangia and obscuring the smooth brown walls of the zygospores.

Cellulis vegetativis 15-18 μ \times 90-180 μ , chromatophoris pyrenoideis 4-8 instructis, monostichis; conjugatione scalae modo; zygosporis, globosis vel ovoideis,

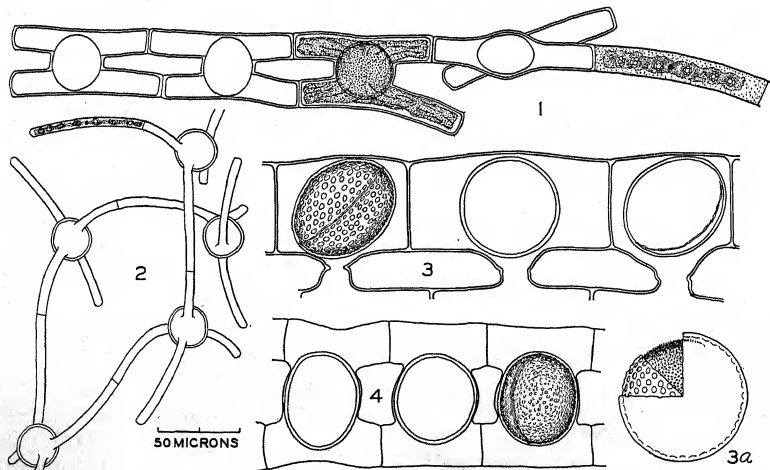


Fig. 1-4.—Fig. 1. *Mougeotia Drouetii*, sp. nov.—Fig. 2. *M. caimani* sp. nov. The irregular orientation of the gametangia is characteristic.—Fig. 3, 3a. *Zygnema crassiusculum* sp. nov.—Fig. 4. *Z. pawhuskiae* Taft.

lateribus compressis, 32–36 μ \times 28–34 μ , tubo completo in gametangia extensis; gametangis post conjugationem membrana fusca granulosaque obsitis, qua membrana zygosporae laevae et bruneae obscurantur.

Brazil: Ceará, Fortaleza. Collected by Dr. Francis Drouet in a pond near Porangaba, October 14, 1935. (Coll. No. 1463.)

This species is the smallest of the group within the genus *Mougeotia*, to which belong *M. sphaerocarpa* Wolle, *M. africana* West, *M. sumatrana* Schmidle and *M. talychensis* (Woron.) Czurdza. (Fig. 1.)

Mougeotia caimani sp. nov.—Vegetative cells 4–5 μ \times 60–100 μ , chromatophores with 2 to 8 pyrenoids in a single row; zygosporae globose or subglobose, 20–25 μ in diameter, surrounded by a thin pectic layer, dividing and enlarging both gametangia. All the zygosporae seen were smooth and colorless.

Cellulis vegetativis 4–5 μ \times 60–100 μ , chromatophoris pyrenoideis 2–8 instructis, monostichis; conjugatione scalari, cellulis conjugalibus obtortis; lamella externa sporangii pectosa; zygosporis grandibus globosis vel subglobosis, diametro 20–25 μ , mesosporio hyalino.

Nearest to *M. cherokeana* Taft, from which it is distinguished by the smaller diameter of the vegetative filaments, the color of the spore wall, and the irregular orientation of the conjugating cells.

Haiti: Treu Caiman, among reeds, February 16, 1933. Collected by Dr. R. M. Bond. (Fig. 2.)

Mougeotia granulosa sp. nov.—Vegetative cells 15–18 μ \times 100–216 μ , chromatophore with 4 to 10 pyrenoids in a single row; conjugation scalariform, sporangium dividing both gametangia; zygosporae mostly quadrate-ovoid, (36–)40–50 μ \times 43–58 μ , angles rounded, sometimes truncate or slightly produced; outer spore wall thin, yellow, granulose, or finely verrucose on the inner side; median wall thick, smooth, and yellow-brown.

Cellulis vegetativis 15–18 μ \times 100–216 μ , chromatophoris pyrenoideis 4–10 instructis, monostichis; conjugatione scalari; zygosporis ovoideis-quadratis 40–50 μ \times 43–58 μ , angulis aut rotundatis aut productis; episporio intus aureo granuloso; mesosporio luteo, laevi et crasso.

Nearest to *M. irregularis* West, from which it differs in the shape of the spores and spore walls; and *M. paludosa* W. & G. S. West, a smaller species with colorless spore walls.

South Africa: Cape Town. Collected by Miss E. L. Stephens on the Cape Flats. Coll. No. 510. Here associated with *Zygnema synadelphum* Skuja and *Zygnemopsis fertilis* (Fritsch & Rich) Transeau. (Fig. 5.)

Spirogyra nyctigama sp. nov.—Vegetative cells 34–38 μ \times (72–)80–140(–180) μ with replicate end walls, one chromatophore making 2–5 turns; conjugation scalariform; tube formed by both cells, receptive gametangium inflated to 65 μ ; zygosporae ellipsoid 47–54 μ \times 80–98 μ , median wall yellow-brown, smooth.

Cellulis vegetativis 34–38 μ \times 80–140 μ , dissepimentis replicatis, chromatophoris singulis, anfractibus arctis 2–5; conjugatione scalari, cellulis fructiferis ad 65 μ inflatis; zygosporis ellipsoideis 47–54 μ \times 80–98 μ , mesosporio laevi et fusco.

South Africa: Cape Town. Collection of Miss Edith L. Stephens No. 1348. (Fig. 11.)

Spirogyra porangabae sp. nov.—Vegetative cells 11–14.5 μ \times 65–145 μ , with plane end walls, one chromatophore making 4–9 turns in the cell; conjugation scalariform with tubes formed by both cells; receptive gametangium enlarged or slightly inflated, sterile cells inflated, often bulliform; zygosporae ellipsoid 21–27 μ \times 47–54 μ , median wall at first smooth, at maturity irregularly but distinctly punctate, yellow-brown.

Cellulis vegetativis 11–14.5 μ \times 65–145 μ , dissepimentis planis, chromatophoris singulis, anfractibus 4–9; conjugatione scalari, tubo ex utraque cellula conjuganda emissio, cellulis sporiferis amplificatis; cellulis sterilibus vel inflatis vel bullatis; zygosporis ellipsoideis 21–27 μ \times 47–54 μ ; mesosporio primo laevi flavescens, deinde fusco et subtiliter punctato.

South America: Brazil. Collected by Dr. Francis Drouet, October 8, 1935, from water weeds in Lake Maraponga, State of Ceará, Fortaleza, Porangaba. Coll. No. 1473.

This species resembles *S. bullata* Jao, but is smaller and does not have smooth spore walls. It differs from *S. perforans* Transeau and *S. minutifossa* Jao in its smaller dimensions and spore wall markings. (Fig. 9.)

Spirogyra maravillosa sp. nov.—Vegetative cells 24–29 μ \times 108–260 μ , with plane end walls, 2 or 3 chromatophores making 2 to 5 turns in the cell; conjugation unknown, aplanosporae broadly ellipsoid, 28–36 μ \times 43–60(–72) μ , median wall smooth yellow-brown; sporangia enlarged or slightly inflated.

Cellulis vegetativis 24–29 μ \times 108–260 μ , dissepimentis planis, chromatophoris 2–3, anfractibus 2–5; conjugatione incognita; aplanosporis ellipsoideis, in apice plus minusve rotundatis, 28–36 μ \times 43–72 μ ; mesosporio laevi, in maturitate flavescens-brunneo.

South America: Brazil. Collected by Dr. Francis Drouet in a bog near Belém, July 9, 1935. Coll. No. 1533.

This species closely resembles *S. mirabilis* (Mass.) Kuetz. but differs in the regular occurrence of 2 or 3 chromatophores. (Fig. 10.)

Spirogyra variformis sp. nov.—Vegetative cells 43–50 μ \times (70–)108–140(–200) μ , with plane end walls, one chromatophore making 2 to 5 turns in the cell; conjugation scalariform, tubes formed by both cells and widest at the middle; some sterile cells inflated to 72(–100) μ ; fertile cells mostly cylindric, but sometimes enlarged and inflated; zygosporae ellipsoid or ovoid, 45–54 μ \times 58–90 μ , rarely spherical 52–60 μ in diameter; median wall brown, smooth.

Cellulis vegetativis 43–50 μ \times 108–140 μ , dissepimentis planis, chromatophoris singulis, anfractibus

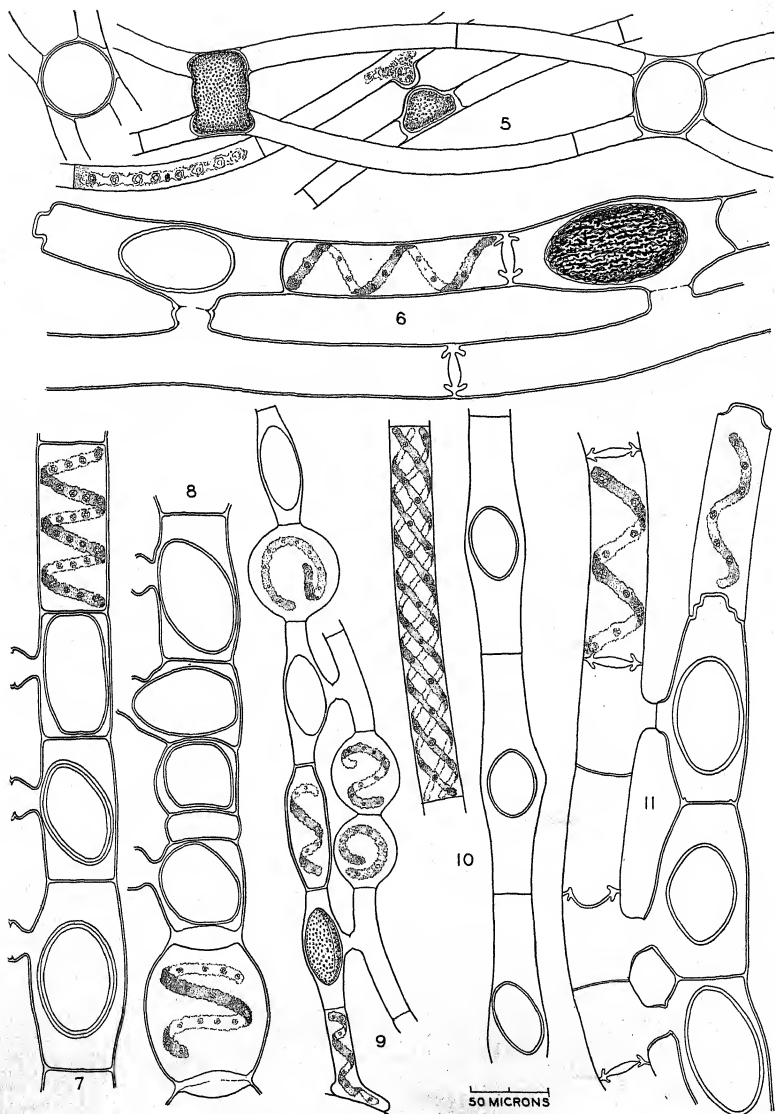
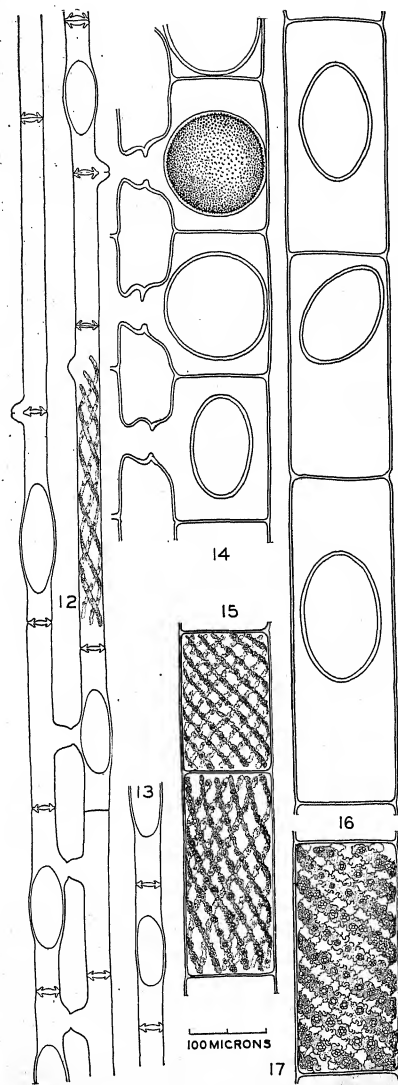


Fig. 5-11.—Fig. 5. Zygospores and a parthenospore of *Mougeotia granulosa* sp. nov.—Fig. 6. *Spirogyra tetrapla* sp. nov.—Fig. 7, 8. *S. variformis* sp. nov.—Fig. 9. *S. porangabae* sp. nov.—Fig. 10. *S. maravillosa* sp. nov., vegetative cell and aplanospores.—Fig. 11. *S. nyctigama* sp. nov.



2-5; conjugatione scalari, cellulis sterilibus interdum tumidis, cellulis fructiferis cylindricis, interdum inflatis; zygosporis ellipsoideis vel ovoideis, $45-54\mu \times 58-90\mu$ episporio crasso et hyalino, mesosporio bruneo laevique.

Africa: Cape Town. Miss E. L. Stephens Coll. No. 46.

This species resembles *S. condensata* (Vauch.) Kuetz. but differs in the variability of the spore form and size. (Fig. 7, 8.)

Spirogyra Wrightiana sp. nov.—Vegetative cells $130-165\mu \times 275-430(-690)\mu$, with plane end walls, 6-8 chromatophores making 1-3 turns in the cell; conjugation unknown; aplanospores mostly ellipsoid, $100-126\mu \times 158-206\mu$; sporangia cylindric and of the same dimensions as the vegetative cells; spore walls in the material seen probably not mature, smooth and colorless.

Cellulis vegetativis $130-165\mu \times 275-430\mu$, dissepimentis planis, chromatophoris 6-8, anfractibus 1-3; conjugatione incognita; aplanosporis ellipsoideis $100-126\mu \times 158-206\mu$; sporangii cylindricis; sporis observatis verisimiliter immaturis, episporio laevi et hyalino.

South America: Brazil. Collected by Dr. Stillman Wright, July 14, 1934, at Campina grande, Paraibo. Drouet Collection No. 2030.

This species approaches *S. hatillensis* Transeau but differs in the complete absence of conjugation and the larger dimensions throughout. (Fig. 16, 17.)

Spirogyra jatobae sp. nov.—Vegetative cells $118-130\mu \times 108-500\mu$, with plane end walls and 8-11 chromatophores, straight or making one turn in the cell; conjugation scalariform, tubes formed by both cells; receptive gametangium slightly inflated, especially on the conjugating side; zygospores compressed spherical, $108-140\mu \times 78-90\mu$, median wall yellow-brown, irregularly and minutely verrucose.

Cellulis vegetativis $118-130\mu \times 108-500\mu$, dissepimentis planis, chromatophoris 8-11, rectis vel anfractibus arcibus singulis; conjugatione scalari; cellulis fructiferis paululum inflatis (quo in latere conjugatio sequitur); zygosporis lentiformibus $108-140\mu \times 78-90\mu$, mesosporio fusco, maturante irregulariter verruculoso.

South America: Brazil. Collected by Dr. Stillman Wright at Jatobá, Pernambuco, October 3, 1933. Drouet Coll. No. 1983.

This species differs from *S. mazima* (Hass.) Wittr. in the character of the spore markings and the number of chromatophores; from *S. jassiensis* (Teodor.) Czarda in the mode of conjugation and the spore markings. (Fig. 14, 15.)

Fig. 12-17.—Fig. 12, 13. *Spirogyra gratiana* sp. nov., showing the characteristic straight filaments in both lateral and scalariform conjugation. Aplanospores and cross conjugation infrequent.—Fig. 14, 15. *S. jatobae* sp. nov., zygospores and vegetative cells.—Fig. 16, 17. *S. Wrightiana* sp. nov., aplanospores and a vegetative cell.

Spirogyra gratiana sp. nov.—Vegetative cells 28–33 μ \times 144–400 μ , with replicate end walls, usually 3 chromatophores (rarely in some cells 2 or 4), conjugation lateral and scalariform, tubes formed by both cells; receptive gametangium cylindric or enlarged; zygospores ellipsoid to cylindric ellipsoid, 35–47 μ \times 108–223 μ , all walls smooth, median wall yellow; aplanospores infrequent, smaller, 38–40 μ \times 47–72 μ .

Cellulis vegetativis 28–33 μ \times 144–400 μ , dissepimentis replicatis, chromatophoris 3 (rarius 2 aut 4); conjugatione laterali vel interdum scalari; tubo ex utraque cellula conjuganda emissio; cellulis fructiferis cylindricis aut paululum inflatis; zygosporis ellipsoideis vel cylindraceo-ellipsoideis 35–47 μ \times 108–223 μ mesosporio laevi, flavescente; aplanosporis cui similibus quamquam raris et minoribus, 38–40 μ \times 47–72 μ .

United States: Minnesota. Collected by Grace Nichols (Mrs. George E. Nichols) in a rock pool on the shore of Lake Superior, three miles southwest of Grand Marais.

This species is remarkable for its straight conjugated filaments and the occurrence of lateral and scalariform conjugation, as well as aplanospore formation. The length of the spores in proportion to their width distinguishes it from all other species approaching these dimensions.

In the same collection occurred *S. foveolata* (Skuja) Czurda and *S. pascheriana* Czurda, which were previously unreported from America, also *Mougeotia pulchella* Wittrock. (Fig. 12, 13.)

Spirogyra tetrapla sp. nov.—Vegetative cells 30–40 μ \times 100–250 μ , with replicate end walls; 1 or 2 chromatophores making 2 to 8 turns in the cell; conjugation scalariform, tubes formed by both cells; receptive gametangia inflated to 66 μ ; zygospores ellipsoid, 48–58 μ \times 68–88 μ , median wall, yellow, made up of 2 layers, of which the outer is thin and irregularly corrugate and the inner finely reticulate.

Cellulis vegetativis 30–40 μ \times 100–250 μ , dissepimentis replicatis; chromatophoris 1 vel 2, anfractibus 2–8; conjugatione scalari; cellulis sporiferis inflatis ad 66 μ ; zygosporis ellipsoideis 48–58 μ \times 68–88 μ , mesosporio aureo duplici, membrana exteriori irregulariter corrugata, membrana interiori subtiliter reticulata.

United States: Illinois, Indiana, Ohio, and Mississippi.

Common in ponds in central Illinois, fruiting during April and May. In my early records confused with *S. areolata* Lagerheim, the spores of which have a double outer wall and a single smooth median wall. (Fig. 6.)

Spirogyra lenticularis sp. nov.—Vegetative cells 150–162 μ \times (80–)125–200(–300) μ , with plane end walls, 9 or 10 chromatophores, straight, or making one turn in the cell; conjugation scalariform, tubes formed by both cells, receptive gametangia shortened and cylindric or slightly enlarged on the inner side; zygospores compressed spheroid, 136–145 μ \times 90–100 μ , outer wall thin, smooth, transparent, median wall thick, minutely verrucose and with labyrinthine reticulations, brown.

Cellulis vegetativis 150–162 μ \times 125–200 μ , dissepimentis planis, chromatophoris 9–10, rectis vel anfractibus singulis; conjugatione scalari; cellulis fructiferis cylindricis, vel paululum inflatis; zygosporis lentiformibus 136–145 μ \times 90–100 μ ; mesosporio crasso bruno, verruculoso et irregulariter reticulato.

South Africa: Capetown. Collection Miss E. L. Stephens No. 1039.

Differs from *S. maxima* (Hass.) Wittr. in the number of chromatophores, in its larger dimensions, and in its distinctly different spore-wall markings. (Fig. 18, 19.)

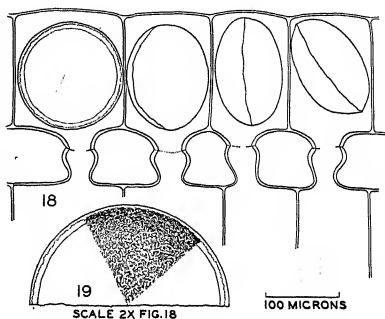


Fig. 18–19.—Fig. 18, 19. *Spirogyra lenticularis* sp. nov., characteristic zygospores and the markings on the median spore wall.

It may be of interest also to record the occurrence of *Zygnemopsis minuta*, recently described by Dr. Randhawa from the Upper Punjab, India, in a bog pond near Douglas Lake, Michigan, collected by Dr. Clarence E. Taft. Among the algae collected by Miss Stephens near Cape Town (Coll. No. 319) is a species recently described by Dr. H. Skuja from Greece: *Spirogyra ophanosculpta*. Specimens of both are in my collection.

In conclusion, I wish to thank the various collectors for their kindness in sending me specimens which have not only yielded new species, but have extended the ranges of many others.

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MIGRATION OF SALTS AND WATER INTO XYLEM OF THE ROOTS OF HIGHER PLANTS

A. S. Crafts and T. C. Broyer¹

DESPITE THE immense amount of research on absorption and movement of mineral solutes and water in plants, the mechanisms of the processes remain obscure. For the teacher and investigator a description of a possible mechanism of migration of solutes and water in roots, based upon modern research and compatible with known root structure, should be of interest.

Recent reviews (Hoagland, 1936, 1937; Steward, 1935) summarize the achievements in the field of plant nutrition. Utilizing single cells, filaments, excised root systems, and whole plants, these studies describe the phenomena of salt accumulation and of the response of plants to their culture solution environment. It seems logical that they should provide clues to the mechanics of salt migration and the part played by growth and development. A tentative mechanism related to known structure and function of plant roots and designed to explain uptake of water and salts which may occur simultaneously² is described.

The relations of absorption to organic food supply, available oxygen, and ion accumulation are reviewed. An attempt is made to evaluate the role played by root pressure and by translocation³ of water and salts.

QUANTITATIVE ASPECTS OF XYLEM EXUDATION.—An excised squash root may exude more than its own weight of sap in 24 hours, replacing many times the contents of its xylem vessels (Crafts, 1936). Collections from excised roots of two 55-day-old Hubbard squash plants grown in aerated full Hoagland's solution (Hoagland and Broyer, 1936b) were as follows: Plant number 1, whose absorbing roots (all tissue below the culture solution level) weighed 81.7 grams fresh and 3.4 grams dry, exuded 243 cc. during the first 24 hours and 100 cc. during the second like period; Plant number 2, with absorbing roots weighing 136.0 grams fresh and 5.8 grams dry, exuded 40 cc. during the first hour, 343 cc. during the first 24 hours, and 140 cc. during a second like period. The nitrate concentration of the xylem exudate was more than twice that of the culture solution during the early stages of the experiment and decreased to an

equal concentration after the first 24 hours. These results show the ability of the plant root to pump water against gravity and to concentrate solutes against osmotic force.

Experiments show (Hoagland, 1936, Hoagland and Broyer, 1936a, and others) that exuded sap from such roots may contain all the mineral elements normally absorbed by plants. Often salt concentrations of exudates exceed by many times those of the culture media (Hoagland, 1936; Hoagland and Broyer, 1936a; Steward, 1935). This evidence, with that from many absorption experiments, indicates that water and solutes may enter the root simultaneously and that, at least under certain conditions, they are released into the xylem vessels together. Concentrations of solutes in exudate and culture medium show, furthermore, that the absorption of solutes and their secretion into dead xylem elements may exceed in rate the movement of water. Where such secretion occurs, it seems evident that the root is performing work, and studies on the relation of respiration to root absorption substantiate this conclusion. The oxygen requirement, the necessity for a supply of organic foods, and the general relation of root growth and activity to mineral nutrition indicate a mechanism dependent upon the vital functioning of living cells. The following presentation indicates a possible process by which the plant may apply energy in the movement of water and solutes into roots.

THE MODEL OF THE PROPOSED PROCESS.—The structure of the root in the absorbing region has certain pertinent characters (fig. 1). Tissues external to the endodermis⁴ have prominent intercellular spaces and thin cell walls. All cells are in approximate contact through walls (Priestley, 1920) with the culture medium. Cells of the endodermis, in its primary condition in this region, have suberized Casparian strips which effectively prevent leakage of solution under pressure from the stele. Tissues inside the endodermis are small-celled and tightly packed and lack intercellular spaces.

Studies on the occurrence of plasmodesmata in living tissues show that where pits occur the protoplasts are commonly connected by these strands. Consequently the "symplast" (see Münch, 1930) of the root must constitute an interconnected protoplasmic unit, and movement of solutes within the protoplasm proper should readily occur to satisfy concentration gradients.

⁴As used here, the term endodermis refers to this structure in its primary condition where suberization of the walls is limited to the Casparian strip. In species having secondary thickening of the endodermis, passage of solutes is gradually reduced until finally, with complete restriction of food movement, the cortex dies and sloughs off.

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The writers desire to express their appreciation to Professor D. R. Hoagland and others of the University of California at Berkeley and at Davis, who, by their helpful suggestions, have aided in the development of this presentation.

²By the term "simultaneously" the writers do not infer that the rates of uptake of water and salt necessarily show any direct interdependence.

³While not excluding possible movement by other routes, it is assumed that under normal conditions the major longitudinal transport of water and salts from roots takes place through the xylem.

In contrast with this protoplasmic unity, anatomical features of the root designate two tissue systems that are physiologically distinct. These are: (1) the cortex, in direct contact with the culture medium and aerated by the culture solution, by the elaborate system of intercellular spaces present, or by both; and (2) the stele, which receives its salts and oxygen from the solution that has passed through the cortex. Cortical cells have the first opportunity to absorb oxygen and mineral nutrients from their environment. Stele tissues have restricted opportunity for absorption and, furthermore, are exposed to products of metabolism of all the growing and absorbing region. They are bound within a cell layer capable of withstanding internal pressure but relatively impervious to oxygen. They surround the dead xylem vessels that constitute the only channels of escape for a solution above atmospheric pressure, and the path of least resistance for movement of sap under tension.

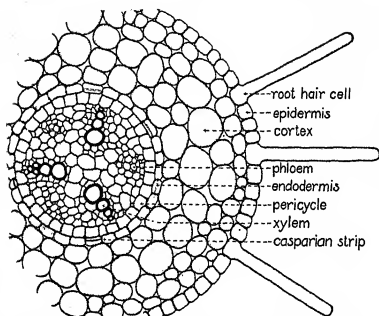


Fig. 1. Semi-diagrammatic view of a transverse section of squash root in the region of absorption.

Apparently the structure of the root imposes upon the cortical cells of the absorbing region an environment favoring maximum growth and accumulation and a high metabolic activity. On the contrary, the living cells surrounding the xylem vessels of this region exist in a different environment—one tending to maintain a lower level of cell activity and to favor, in comparison with the cortex, loss of solutes. Protoplasmic continuity should, by protoplasmic streaming and diffusion, tend to maintain equal concentrations of ions across these layers. The net result should be that, following absorption in the cortex, ions pass inward along the protoplasm in concentrations that cells inside the Casparian strip cannot maintain, resulting in a loss into the xylem.⁵ Structure indicates

⁵ Since permeability is a property of protoplasm conditioned by the cumulative effects of environmental factors and causally related to activity, any change in metabolic activity-permeability relations (caused by environmental or growth controlling factors, stimuli, or repressants) should modify rates or equilibria of the vital

the occurrence of a gradient of decreasing O_2 and increasing CO_2 from the cortex of the root to the stele. Absorption and exudation experiments are not inconsistent with an activity gradient in the protoplasm of the root paralleling this O_2 : CO_2 gradient and presumably conditioned by it.

THE MECHANISM OF THE PROPOSED PROCESS.—Following the above consideration, it is proposed that the migration of salt takes place as follows. The uptake of ions from the culture medium occurs in the cortical region, where the capacity of the cells to hold solutes is at a maximum. Diffusing inward along the protoplasm, they pass from cell to cell until they arrive in the stele where, because of the relatively low activity, the protoplasm is not in a condition to retain them at a concentration equal to that in the cortex. As they leak from the protoplasm into the cell walls of the stele, they maintain across the endodermis an osmotic gradient. Because all intervening cells have an accessible moisture supply, water must move in to satisfy the gradient between the soil or culture solution and the sap within the stele, resulting in the development of a solution under hydrostatic pressure within this region (Blackman, 1921; Heyl, 1933; Ingold, 1935; Priestley, 1920; Sabinin, 1925). Seeking the pathway of least resistance, this solution follows the walls to the xylem vessels, where it accumulates and moves up through the plant.⁶ Leakage to the exterior is prevented by the impervious Casparian strip. It is this solution, less concentrated than the vacuolar sap of the cortical cells but more concentrated than the soil solution, that appears at the cut end of the excised root system in the form of xylem exudate. That it is not an abnormal flow resulting from cutting can readily be shown by placing healthy culture solution plants in saturated atmosphere. As soon as existing deficit is satisfied, solution of like concentration and in like volume appears as droplets at the hydathodes and drips from the plant.

processes of accumulation and migration. Regardless of such changes, the mechanism of the process, as described below, should continue to function, though rates and volumes do vary.

⁶ Though the hydrostatic pressure developed by this system might, as stasis is approached, attain a value approximating the force by which plants are able to take water from soils (16 to 20 atmospheres) (see White, 1938), under normal conditions of transpiration most plants with expanded leaves move water upward through the xylem under pressures less than atmospheric. Active movement of xylem sap by the mechanism here proposed is probably limited to seedlings, plants growing in a saturated atmosphere, and trees devoid of leaves. That such movement is not essential to the rise of sap in tall trees is indicated by the fact that our tallest species are evergreen and seldom, if ever, exude xylem sap. That the system is essentially osmotic may be demonstrated by placing roots of culture solution plants in hypertonic sucrose solution; the leaves soon wilt, and cut stems absorb eosin, which is rapidly drawn back into the roots (Kramer, 1932; Tagawa, 1934). (See the use of this phenomenon in the acid-arsenical method of weed control (Kennedy and Crafts, 1927; Crafts, 1933).)

Hoagland (1936) has emphasized that the problems of absorption, translocation, and exudation by roots involve two factors. These are the accumulative capacities of the living cells as conditioned by their salt status and metabolic activity, and the absorption and passage into the xylem vessels⁷ of solutes. Concentrations of various ions in the exuded sap need bear no obvious relation to those of the culture medium (Hoagland, 1936), the amounts and proportions of ions released depending upon relative rates of absorption and the ability of the cells of various tissues to retain them.

Rather than depending upon differential permeability resulting from a gradient in acidity as postulated by Priestley (1920), the performance of the mechanism proposed here results from differential activity along a gradient of decreasing O_2 and increasing CO_2 . Such a mechanism gives a more rational explanation for the demonstrated need for oxygen and organic foods. It relegates to a minor position the osmotic effects of materials released from differentiating xylem vessels. Placing the bulk of the burden of accumulation upon the highly active cells of the cortex, it seems to be the only mechanism so far proposed that will at the same time account for the volume and solute concentration of exudate collected from certain experimental plants.

Recent works by Hoagland and Broyer (1936b), Prevot and Steward (1936), and Steward (1935) stress the importance of oxygen in absorption by root systems. They indicate the function of living cortical cells, the effects of previous treatment, and the relation of age and position of cells in the organized root to absorption. Early experiments on permeability and recent ones on gas storage and solute accumulation have shown the deleterious effects of high CO_2 and low O_2 concentration upon the activity and consequently upon the ability of cells to attain and maintain high accumulations of solutes. (See Hoagland and Broyer, 1936b; Steward, Berry, and Broyer, 1936.)

If migration of salts in roots involves an activity gradient in their tissues, the essential features of the proposed mechanism should be reconcilable with known root structure and response, and certain of the functional relations should be subject to quantitative determination. Structural relations, on the other hand, do not readily submit to quantitative experimentation, and the criterion for judging these depends upon the degree to which the known structures fit the mechanical requirements imposed by the functions performed. Final acceptance of any mechanism must rely, therefore, not only upon chemical analyses but also upon anatomical studies and finally upon an integration of all such information with respect to the functioning unit. A few of the more relevant issues in the problem will be discussed.

OXYGEN SUPPLY.—There are three obvious sources of oxygen to roots: (1) the soil, including the soil

atmosphere and dissolved oxygen in the soil solution; (2) the shoot system of the plant, provided it is closely enough connected so that conduction through the cortical system of intercellular spaces (fig. 2) may be effective; and (3) the anion supply consisting principally of absorbed nitrate and secondarily of sulfate.

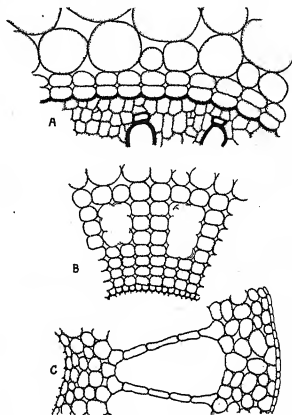


Fig. 2. Cortical tissues showing varying degrees of specialization to facilitate aeration.—A. Corn root above the absorbing region. Intercellular spaces are prominent in the cortex. The endodermis is undergoing secondary thickening.—B. A more mature region than A, showing breakdown of cortical cells resulting in elongated passageways.—C. Rhizome of *Myriophyllum* showing highly specialized passageways in the cortex.

The oxygen-supplying power of the soil has long been recognized, and horticultural practices have included drainage and tillage designed to aerate the soil. Porosity, temperature, and biological activity by microorganisms largely determine the amount of oxygen available to higher plants and may limit the agricultural value of a soil.

The importance of the "internal" supply of oxygen has been pointed out by Cannon (1932) and will undoubtedly receive increasing attention as the significance of root respiration is more widely recognized. There seems little doubt that the oxygen released into the intercellular spaces of the leaf as a result of photosynthesis or entering from the air may move through the cortex of the petiole and stem and reach the root system. A consideration of the solubility relations of O_2 and CO_2 indicates that in aquatics an actual pressure flow may be established. The well-known evolution of oxygen by *Elodea* is the direct reverse of this process. In *Elodea* shoots, carbon dioxide in solution is absorbed, and the rela-

⁷ See footnote 3.

tively insoluble oxygen produced in like volume is released in gaseous form. In the root the gaseous oxygen is absorbed, creating a reduced gas pressure in the intercellular space system of the cortex. Gas from the leaves must immediately move down to satisfy this deficit, resulting in an effective pressure flow. The carbon dioxide produced is released into the cell walls and either diffuses into the soil or culture solution or is swept along into the stele by the moving water. Rapid photosynthesis provides both oxygen and carbohydrates for use by the roots.

That the reduction of nitrate in roots may provide oxygen for respiration has not been widely recognized. Recent work by Arnon (1937) indicates the importance that this supply attains when comparisons are made of ammonia and nitrate as sources of nitrogen. Barley plants growing in culture solutions of C.P. salts with ammonia as a nitrogen source required either forced aeration or a supplement of certain micro-elements, particularly Mn or Cu, to produce yields comparable to those of plants grown in solution containing nitrate.

The relation between these three oxygen sources is of interest. Cannon (1932) found with roots in water that oxygen absorption from the culture medium was practically stopped during periods of illumination. In the absence of solute absorption the "internal" supply was adequate for respiration.

When Arnon (1937) supplied nitrate to barley plants in shallow water culture, forced aeration was not required in the presence of solute absorption. The internal supply plus the anion supply adequately supplemented absorption from the atmosphere even for the higher respirational level of absorbing roots. When the nitrogen was supplied as ammonia, however, aeration or an adequate supplement of certain heavy metals was required. Although the exact effect of these micro-elements is not known, they apparently affect respiration. Either they catalyze oxygen absorption at lower oxygen tension, they increase the efficiency of oxygen utilization, or in some way they stimulate synthetic processes so that the "internal" supply of oxygen is increased.

Hoagland and Broyer (1936b) and Prevot and Steward (1936) have shown that excised root systems having no "internal" oxygen supply require forced aeration for optimum absorption of salt. Potato root tissues used by Steward, Berry and Broyer (1936) required aeration with an atmosphere containing approximately 10 per cent oxygen for maximum rate of absorption, while the barley roots used by Hoagland and Broyer were able to accumulate some salt, although the percentage of oxygen was reduced to 5 per cent or even less (Hoagland and Broyer, 1936b).

Certain plants, notably squash and cotton, as used by Hoagland and Broyer, respond to forced aeration in culture solutions containing nitrate and the supplementary solution containing micro-elements. Although cereals may respond to forced aeration by changes in root form, this change is not necessarily attended by an increased yield. Cereals consequently do not ap-

pear to correspond in oxygen requirements with squash and cotton, while plants that do are so developed that the principal photosynthetic surface is farther removed from the roots. Though this phase of the story is far from complete, there seems to be some evidence that differences in response to increased oxygen supply may be partly explained on the basis of structure. The requirement for aeration, for instance, may relate to the adequacy of the "internal" supply as affected by the length and form of the intercellular channels of the cortex. A notable development of the cortical aeration system is observed in the roots of many water plants (fig. 2C).

The oxygen supply derived from nitrate may be an important source of oxygen to deeply-penetrating roots of some plants, especially during rapid growth in the spring. Nitrates leached into the subsoil by winter rains would thus constitute an important factor in root penetration and consequently in plant distribution. The presence of reduced sulfur in proteins and of organic compounds containing phosphorus in xylem exudate (Pierre and Pohlman, 1933) indicates a similar function for these elements.

ION UPTAKE.—The complex inter-ionic relations of accumulation by root cells lie outside the subject matter of this paper. It suffices to emphasize the parallelism between ion uptake and root exudation pointed out by other investigators (Hoagland, 1936; Hoagland and Broyer, 1936a; Laine, 1934; Skoog, Broyer and Grossenbacher, in preparation). The immediate problem is to explain the secretion of ions into the dead xylem vessels after their absorption by cortex cells. When this secretion has been accomplished the movement of water under conditions of low transpiration should take place to satisfy osmotic gradients. The relative abundance of the various ions in the xylem sap is a complex matter of accumulation capacities, mobilities, and ion penetrability and there is little evidence that it affects the end result except as it determines osmotic concentration.

ORGANIC NUTRIENT SUPPLY.—Since the ability of roots to accumulate ions depends upon energy from foods, a high rate of migration through root tissues would necessitate an ample food supply.

Although in the organized root system organic nutrients move down in the phloem and become distributed via the protoplasm (Crafts, 1933a), experiments by Prevot and Steward (1936) and White (1936) indicate that an external supply is also effective. The power to accumulate and translocate carbohydrates attributed to phloem parenchyma (Crafts, 1933a) may be common to all parenchyma, the supply rather than the exact structure being essential. This indicates that the root pressure mechanism of Münch (1930) does not present the correct picture.

WATER MOVEMENT.—It seems evident that water absorption by roots takes place under widely differing conditions of pressure. When uptake exceeds transpiration loss, the xylem sap is under positive pressure, and water absorption is accomplished by

the root with an appreciable expenditure of energy. Under these conditions water movement is relatively slow, taking place, according to the proposed mechanism, along an osmotic gradient between the culture medium and the xylem. Any factor tending to increase the concentration of solutes in the xylem would accelerate water uptake. As Hoagland and Broyer (1936a, 1936b) have shown, rapid exudation accompanies rapid ion absorption.

When transpiration exceeds water uptake, the pressure conditions change; the root tissues assume a more or less passive function; and water movement is less dependent upon solute absorption. Cannon (1932) has shown that rapid transpiration increases oxygen absorption by roots during the day, a fact that might allow for increased solute absorption. His results show, however, not oxygen utilization but differential absorption as affected by the "internal" supply; and since his data do not cover photosynthesis and solute absorption, the actual metabolic activity of his roots remains unknown (Henderson, 1934). Under all conditions, however, water absorption apparently results from movement along a water activity gradient, transpiration pull tending to reduce the activity of water in the xylem even more effectively than do the solutes that are released by root cells. Solute uptake, on the other hand, depends upon supply and a protoplasmic activity gradient and would be influenced by water movement only insofar as the latter affected concentration gradients, or activity, by accelerating the movement of gases into the stele.

Furthermore, it seems possible that a rapid removal of the available soil moisture as a result of active transpiration would present ions to the absorbing surfaces of the root in higher concentration than when transpiration is low. This maintenance of supply may actually increase absorption of salt, at least under certain conditions (Freeland, 1935; Hoagland and Broyer, 1936a).

Although absorption of water and ions would therefore be largely independent, there is no apparent reason, anatomical or physiological, for assuming, as Curtis has done (1935, p. 78-79) that a partition of mineral nutrients takes place between xylem and phloem in the root. The presence of both organic and inorganic nitrogen compounds in the xylem sap, as well as other mineral nutrients (Anderson, 1929; Hoagland and Broyer, 1936a; Pierre and Pohlman, 1933) indicates that these materials move in the transpiration stream; the relative abundance of either seems related to the conditions existing in the functioning roots at the time of absorption.

SECONDARY MOVEMENTS OF SOLUTES.—The relation of primary to secondary movements of materials in vascular tissues must depend upon the relative activities of the functioning tissues. Organic radicals acting in nitrate reduction in the roots, for instance, depend upon the relation of available sugars to nitrate absorbed (Leonard, 1936). In trees where absorption and movement are relatively slow, most of the trans-

located nitrogen is in organic combination (Thomas, 1927). Lack of oxygen in the atmosphere surrounding the roots of trees may be another factor in this phenomenon. Nitrogen, when absorbed as nitrate in shallow-rooted herbs, may be largely moved as such. This occurs in tomato when copiously fertilized with nitrate. In other plants—for instance, cotton (Mason and Maskell, 1931) and sunflower (Leonard, 1936)—both nitrate and reduced nitrogen compounds move in the transpiration stream. The fate of the nitrogen compounds depends ultimately upon the ability of the shoot to retain them. If this is low, they may enter the phloem stream and again find their way into the roots (Maskell and Mason, 1929). In the long-time economy of plants, however, this is probably unimportant. The primary-shoot meristems and the vascular cambium are the principal tissues benefited by such nitrogen compounds.

GENERAL ASPECTS OF SALT MIGRATION IN ROOTS.—The foregoing discussion emphasizes the complexity of the functioning root system of the higher plant and points out some relations of the factors whose interplay results in absorption and migration of salts. Probably its greatest value lies in the consideration given to the roles of these various factors as they have been determined by previous investigators and in the suggested effects of structure upon the activities of different tissue systems in the root. The relation of root anatomy to the supply of oxygen and to the elimination of carbon dioxide, as these affect activity, seems inescapable.

The process of migration seems definitely associated with the process of accumulation by root cells; much modern research emphasizes this association. The ultimate liberation of solutes into the dead xylem elements, however, involves in addition a process which, for lack of a better name, may be called secretion. Some physiologists would identify this secretory activity with a differential accumulative capacity at the outer and inner sides of root cells, and some have attempted to relate such polar secretion to electrical gradients within the root. Obviously, however, it is difficult to determine the cause-and-effect relations of electrical effects in a root having demonstrated concentration gradients of electrolytes.

The thesis of the present paper is that structural relations of the root impose upon the tissues certain environmental conditions which, in turn, normally regulate activities. That the external tissues accumulate solutes and the internal tissues lose them seems inevitable if interpretations of the effects of the O_2 : CO_2 gradients upon activity are permissible.

The actual movement of solutes from cell to cell within the root is assumed to occur by diffusion, accelerated by protoplasmic streaming except along plasmodesmata where streaming has not been demonstrated.

Observed rates of uptake and exudation seem compatible with this mechanism. The presence of bromide ion in xylem exudate of squash within 30 minutes after exposure of the roots to solution con-

taining 800 ppm. KBr indicates one measured rate. Assuming a rate of streaming of 2.0 cm. per hour (Crafts, 1933a) and a distance of 0.5 mm. to be traversed from root hair to xylem, $1\frac{1}{2}$ minutes would be consumed in carrying ions about 99 per cent of the distance from the culture medium to the xylem vessels. With upward flow at 1 cm. per minute, another 10 minutes would be required to move them to the open ends of the vessels. This would leave $18\frac{1}{2}$ minutes for diffusion of the ions through the .005 mm. of protoplasm traversed in their movement through living cells. This seems possible in view of the concentrations involved, but little is known of actual rates of diffusion through living membranes.

That a concentration gradient within the protoplasm should parallel the gradient in activity seems logical. In the final analysis, however, this mechanism requires that the system *cortex protoplasm-endodermis protoplasm-stele protoplasm* constitutes a continuous phase differing in properties from the system *external solution-protoplasm-vacuole*. If this difference does not occur, one cannot explain the movement of ions along the "symplast" from cortex to stele. Each cell would accumulate to its maximum capacity, depending upon its individual environment; and the xylem sap, if it contained more solutes than the culture solution, would constitute a more available source and hence would lose rather than gain solutes.

The choice,⁸ apparently, must lie between some

⁸ Let it be emphasized here that this paper attempts to explain only the process of migration of water and salts into roots on the basis of known root structure. Other processes of unilateral secretion, such as the polar distribution of sugar in the foliage leaf (Phillips and Mason, 1933), the secretion of salt across a leaf under illumination (Arens, 1936) reported by Gregory (1937),

structural polarity, involving a differentiation between cortex and stele, and, the endodermis excepted, a cellular polarity wherein the two sides of the individual cell differ. In making his choice, the investigator should not neglect the fact that root anatomy imposes an environmental difference upon tissues inside and outside the endodermis. That this environmental difference causes the physiological gradient essential to the mechanism here proposed seems logical.

SUMMARY

The problem of salt migration in roots of higher plants as contrasted with accumulation by cells is posed, and a mechanism to explain solute migration, water movement, and root pressure is suggested.

The various activities of the root are discussed, including oxygen supply, ion uptake, organic nutrient supply, water movement, and secondary movements of solutes. The relations of these factors to this mechanism are pointed out, and supporting evidence from the literature is cited.

A mechanism depending upon an activity gradient, conditioned by environmental differences imposed upon the tissues by their very structure, seems best to explain observed salt migration in roots. Such a mechanism fits most of the data and is consistent with known root structure.

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and production of latex, resins, and other such materials, are not considered.

Accumulation of ions within the protoplast is an integral part of the mechanism of the process described; it is assumed to be a commonly accepted phenomenon and no attempt is made here to explain its mechanics.

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AFTER-EFFECTS OF THE TREATMENT OF SEED WITH AUXIN¹

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IT HAS been shown repeatedly in the last few years (see Went and Thimann, 1937) that certain concentrations of auxin, such as promote growth in shoots, cause an inhibition of the elongation of roots. The action is completely non-specific, both as to type of plant and type of auxin. Thus, roots of *Agrostemma* (Meesters, 1936), *Avena* (Kögl, Haagen-Smit, and Erxleben, 1934; Lane, 1936), *Lupinus* (Faber, 1936), *Pisum* (Thimann, 1936), *Vicia Faba* (Faber, 1936), and *Triticum* (Marner, 1937) all show the same effect. Among the auxins, auxentriolic and auxenolonic acids (auxin *a* and *b*), indole-acetic, -propionic, and -butyric acids, indene-acetic, and allocinnamic acids all are effective, though over different concentration ranges, so that root inhibition is doubtless a general property of all substances having auxin activity.

On the other hand, it has recently been made clear that in the presence of extremely dilute auxin solutions roots are slightly accelerated in their growth, and this is confirmed in the paragraphs immediately below. The relation of root growth to auxin concentration is therefore an optimum curve, as discussed by Thimann (1937). This fact has an important

bearing on the experiments to be discussed in this paper.

In none of the previous experiments in this field has the subsequent behavior of roots which have been inhibited by auxin been followed. The present experiments have started from the observation that such roots, on transference to a medium free from auxin, are afterwards accelerated in growth rate. This, and the accompanying effects on the growth of the shoot, may be considered as after-effects of the auxin treatment.

STIMULATING EFFECT OF VERY LOW AUXIN CONCENTRATIONS ON ROOT ELONGATION.—Oat or wheat seeds were husked, soaked, laid out on wet filter paper for forty-eight hours, and then transferred to filter paper in 10 cm. Petri dishes, each of which contained 6 cc. of the solution to be tested. Serial dilutions of pure re-crystallized indole-3-acetic acid down to 10^{-6} mg. per liter (5×10^{-12} M.) were used. All experiments were carried out in the dark room at 24° with occasional red light. Since it has been shown by Lane (1936) that during the period of auxin inhibition the length of the longest root is a constant fraction of the length of the total root system, the longest root was measured from time to time up to the eighth day—i.e., during five days in the auxin solutions.

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Table 1 shows the length of the longest root of *Avena sativa* (Segerhavre or Victory oats) and table 2 similar data for *Triticum* (winter wheat), which in this case was exposed for only four days to the auxin solution.

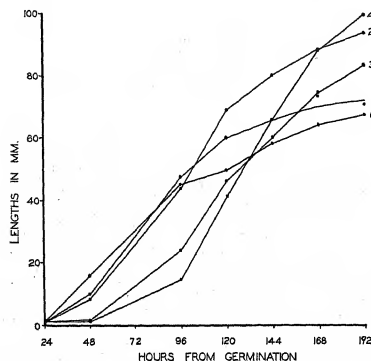


Fig. 1. Length of longest root of *Avena*. Plants treated with indole-acetic acid for the first 24 hours after germination, then transferred to water; kept in dark room throughout. Curve 0, control, no auxin. Curve 1, 0.0013; curve 2, 0.80; curve 3, 20.0; curve 4, 200.0 mg. indole-acetic acid per liter. Experiment of Feb. 28, 1936.

Both tables show that the elongation of the roots is increased by the dilute auxin solutions, the increase being in *Avena* as high as one-third the length of the water controls. The optimum auxin concentration is 10^{-3} — 10^{-4} mg. per liter, which agrees satisfactorily with the findings of Fiedler (1936) (10^{-4} mg. per l.) and of Amlong (1936) (2×10^{-4} mg. per l.) on *Zea mays* and *Vicia Faba*, respectively. Geiger-Huber and

TABLE 1. Longest root of *Avena sativa*. Plants in dark room at 25°. Mean of 15–20 plants in each group.

Auxin concentration in mg. per liter ..	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Days from soaking	Root lengths in millimeters					
3	25	22	34	24	33	23
4	41	50	48	48	42	41
5	57	57	67	81	80	66
8	98	108	114	130	122	106
Increase over controls		10%	16%	33%	24%	8%

Burlet (1936) found a somewhat lower optimum for sterile *Zea mays* roots in culture solution. This lower optimum is doubtless due to the fact that under the above conditions considerable inactivation of auxin occurs, which is at least in part bacterial, so that under sterile conditions the activity of dilute auxin solutions would be better maintained.

TABLE 2. Longest root of *Triticum*. Plants in dark room at 25°. Mean of 15–20 plants in each group.

Auxin concentration in mg. per liter	0	10^{-5}	10^{-4}	10^{-3}	10^{-1}
Days from soaking	Root lengths in millimeters				
3	35	33	33	32	27
6	86	86	87	89	87
8	101	110	115	117	110
Increase over controls		9%	14%	18%	9%

AFTER-EFFECT OF HIGHER AUXIN CONCENTRATIONS ON ROOT ELONGATION.—If the plants are treated, either at the age of twenty-four or forty-eight hours, with more concentrated indole-3-acetic acid solutions, the root elongation is considerably inhibited, as has been previously shown. Inhibition also occurs if the seeds are soaked in the auxin solutions and allowed to remain there twenty-four to forty-eight hours. Under these conditions roots in 200 mg. indole-3-acetic acid per l. grow only one mm. in forty-eight hours, while the water controls grow about 16 mm. However, if at this point the plants are transferred to water, the inhibited roots grow at a gradually increasing rate until their length actually exceeds that of the controls. Figure 1 shows such an experiment with *Avena*. It will be seen that the roots which were least inhibited were the first to be accelerated and those inhibited most were the last—i.e., the treated roots reach and pass the controls in order of increasing concentration of the auxin with which they were treated. Further, the final length reached in all cases was more than that of the controls, the largest increase being 55 per cent. The slopes of the curves show that all the treated roots for a time grow faster than the highest rate reached by the controls and that the maximum rate reached is greatest in the roots pretreated with the highest concentration. Thus, during the 72-hour period from the 96th to the 168th hour, the average growth rates were as follows: control, 0.26 mm. per hr.; 0.0013 mg. per liter, 0.35 mm. per hr.; 0.80 mg. per liter, 0.61 mm. per hr.; 20.0 mg. per liter, 0.69 mm. per hr.; 200 mg. per liter, 1.01 mm. per hr.

The same effect is obtained by very short time treatments, as shown in table 3. Here the seeds were germinated twenty-four hours on filter paper and then treated three hours with the indole-3-acetic acid solutions. On the first day after treatment considerable inhibition was observable, but by the sixth day all the treated plants were accelerated. The effect is particularly large if all the roots are taken into account. It will be noted from the data that the weight per unit length of the roots is also considerably increased. Since this increase here accompanies increase in length, this provides additional evidence (cf. Thimann, 1937) that the weight per unit length is quite independent of inhibition.

AFTER-EFFECTS OF HIGH AUXIN CONCENTRATIONS ON ROOT NUMBER.—The plants treated with high auxin concentrations subsequently produce an in-

creased number of roots (table 4). For this effect to be marked, the auxin treatment should be for twenty-four hours or more; plants treated only three hours show little increase in root number.

TABLE 3. *After-effects of three hours' treatment with auxin solutions in Avena. Mean of 15 plants in each group.*

Auxin concentration in mg. per liter	0	0.01	1.0	100
Length of longest root in mm. 24 hrs. after treatment	17.4	17.1	13.6	4.3
6 days after treatment ..	97	121	125	101
The same as %	100	125	128	104
Total length of all roots in mm.	2628	3390	4055	3102
The same as %	100	129	154	118
Total dry weight of all roots in mg. (rounded) ..	29	40	52	42
The same as %	100	138	179	145
Weight per unit length, mg. per mm.	0.178	0.209	0.210	0.218

Some idea of the large numbers of roots so produced may be gained from figure 2, in which, however, many of the twenty or more roots per plant are at right angles to the plane of the paper and hence are not visible.

TABLE 4. *Avena seedlings treated for 24 hours with auxin solutions. Total number of roots per plant after six days. Mean of 10-12 plants in each group.*

Auxin concentration in mg. per liter	0	1	100	250	500
Average number of roots	5.6	4.7	7.9	17	>20

If the plants are first grown in water for about forty-eight hours and then treated with 100 mg. auxin per l., the elongation of the roots is arrested almost completely during the auxin treatment; if, after twenty-four hours' treatment, they are transferred again to water, elongation commences and the roots show little inhibition. The same thing was shown for a one-hour auxin treatment by Nielsen (1930). An interesting phenomenon may be observed under these circumstances—namely, that some days later a large number of lateral roots appear close to the point of auxin application (see fig. 3).

Thus treatment with high auxin concentrations increases both the number of secondary roots and also, under other conditions, the number of laterals.

AFTER-EFFECTS OF AUXIN TREATMENT ON THE GROWTH OF THE SHOOT.—*Avena*.—When the seedlings treated with auxin are planted out in sand in the greenhouse, the growth of the shoots of the auxin-treated plants shows marked acceleration. This acceleration is first visible, under the conditions used,

about two weeks after planting, and increases with the concentration of auxin used in the pretreatment. It also increases with the time of contact of the seedlings with the auxin solution. In figure 4 are shown plants from seeds swollen in the auxin solution and left in it for forty-eight hours. Only plants treated with the highest concentration (100 mg. per l.) and the water control are shown; the plants treated with the lower concentrations behaved intermediately. The leaves may be seen to be not only longer, but also wider (up to 50 per cent or more) in the treated plants. The haulms are also markedly thicker.

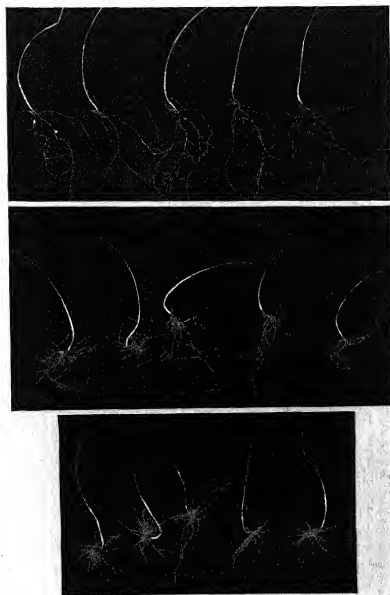


Fig. 2. Root systems of *Avena*. Top, control, no auxin; center, 200 mg. indole-acetic per l.; bottom, 1000 mg. indole-acetic per l. Plants nine days old, in auxin for 72 hours. The water controls have 5-6 roots at this age, the plants treated with the highest concentration averaged 36.

Another experiment carried out in the spring (1936) and another in the fall and winter of 1937 gave precisely similar results. Measurements of the leaf length on this last experiment are given in table 5. They show that (with the possible exception of the lowest concentration) all the treated plants have longer leaves than the controls and that the effect increases

with increasing auxin concentration. Not only is the leaf length increased (and also the width), but the time of appearance of each leaf is hastened. Thus at the sixth leaf (plants six weeks old) the plants treated with the highest concentration were one full leaf ahead of the controls.

TABLE 5. Length of *Avena* leaves measured from soil to tip of leaf. Mean of 10 plants. Experiment of October–November, 1937.

Auxin concentration for seed treatment in mg. per liter	0	10 ⁻⁴	10 ⁻²	1.0	100
First leaf	156	161	159	172	176
Second leaf	214	224	211	223	238
Third leaf	135	141	171	171	196

During two summers a plot of treated plants has been grown outdoors. In each case 100 seeds in each group were soaked in the auxin solution, laid out on filter paper in the same solutions, and planted after three days. In both experiments the treated plants were taller and thicker than the controls, and the effect increased with increasing auxin concentrations. The final height and the weights of straw for the 1937 experiment are given in table 6.

Since in all cases about 100 seeds were used, it will be seen that the germination was incomplete. How-

TABLE 6. Final heights of flower stalks and the dry weights of straw in 1937 *Avena* outdoor crop. Planted May 20, measured and harvested September 7, 1937.

Auxin concentration for seed treatment in mg. per liter	0	1	10	100
Number of plants	70	70	102	91
Mean height in cm.	62.4	67.7	69.7	81.0
Total dry weight of straw	48.1	61.1	93.3	100.3
Mean dry weight of straw per plant	0.687	0.764	0.914	1.103

ever, the treatment with auxin markedly increased the number of plants which germinated. This phenomenon has been observed in a number of experiments with different plants. Apparently treatment of seed with auxin (up to 10 mg. per l.) greatly increases percentage germination. A further observation on some of the experiments has been that the heads of the treated plants were increased in size. The heads of the plants of a greenhouse experiment of the spring of 1938 are shown in figure 5. They show about a 50 per cent increase in the number of spikelets on the treated plants. The full data of this experiment, typical of a number carried out under greenhouse conditions, are given in table 7. It will be noted that although the treated plants flowered a few days before the controls, they had nevertheless formed one more leaf.

TABLE 7. Victory oats treated with indole-acetic acid, 100 mg. per liter, for 24 hours after soaking, then planted in soil. Controls treated with water for the same time. Started Oct. 20, 1937. Grown in greenhouse.

	Treated	Control
Number of leaves at age 3 weeks	2.9	2.0
Number of leaves at flowering	14.4	13.5
Date of first flowering	Feb. 23	March 1
Date of last flowering	March 1	March 3
Average number of spikelets per head	25.2	17.4
Average dry weight of root system (washed free from soil and dried to constant weight)	880 mg.	560 mg.

Lycopersicum.—A number of experiments were carried out with commercial tomato (Comet variety). These will not be given in detail. Plants from seeds swollen in auxin solution (100 mg. per l.) and laid out on filter paper with the same solution for three days showed little difference at first from the controls treated the same way in water. However, after some weeks in the greenhouse the treated plants had become noticeably superior to the controls. Just as with *Avena*, an important part of the difference is in the leaf area. As a measure of the effect, the lengths from the point of insertion of the cotyledons to the extreme top of the first five leaves are given in table



Fig. 3. Root systems of *Avena*; coleoptiles removed before photographing. Left, control, no auxin; right, 3 plants transferred to indole-acetic acid 100 mg. per liter at 48 hours after germination, and after 24 hours in the auxin returned to water for a further 72 hours. Note the knot-like branching induced and the continued elongation of the main root after removal of the inhibition.

8, together with the widths. The lengths were measured to the cotyledons because the bases of the leaves are inaccessible when they are so young. In Comet tomatoes the internodes at this age are very short. The increases in leaf size are proportional throughout, as measurements of individual leaflets in table 9 show. While it is difficult to obtain numerical values on leaves of such complex shape, it is clear from tables 8 and 9 that the auxin treatment hastens the development of the photosynthetic area at any given time by 50-90 per cent.

TABLE 8. Length from cotyledons to tip of leaves and maximum width of tomato leaves. Treated November 18-21, 1936; measured January 20, 1937. Mean of 18 plants in each group.

	LENGTH		
	Water	Auxin (100 mg. per l.)	% increase
Leaf 1	57.7	61.5	6
Leaf 2	70.6	73.7	4
Leaf 3	61.5	72.5	18
Leaf 4	50.0	59.3	19
Leaf 5	30.1	35.6	18

	WIDTH		
	Water	Auxin (100 mg. per l.)	% increase
Leaf 1	36.3	39.4	11
Leaf 2	45.3	52.1	15
Leaf 3	42.5	52.1	23
Leaf 4	33.5	44.2	31
Leaf 5	19.0	24.9	31

The conditions under which this stimulating effect is obtained with tomato are not fully understood, for other series of experiments have shown very little effect. The difference is not due to length of day, as was shown by imposing fixed day-length on half of

week earlier than the controls. The same was true of the tomatoes; on March 1, 1937, the number of flower buds visible on sixteen control plants was 45, on the treated plants 109. However, this effect on tomatoes was not obtained consistently. The principal action of auxin is apparently on the vegetative growth; this agrees with all that is known of auxin

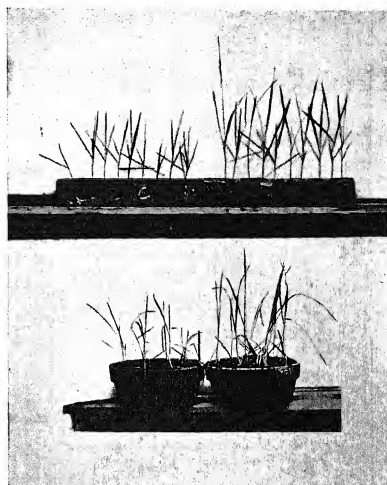


Fig. 4. *Avena* plants grown in the greenhouse from treated seed. Left, control, no auxin; right, treated with indole-acetic acid 100 mg. per liter for the first 24 hours only. Upper photograph, 2 weeks old; lower photograph, 5 weeks old. Experiment of Dec. 1935-Jan. 1936.

TABLE 9. Length and width of leaflets. Same plants as table 7 measured nine days later. Mean of 18 plants in each group.

	Length in mm.			Width in mm.		
	Water control	Auxin	% increase	Water	Auxin	% increase
Leaf 4:						
2nd leaflet	20.5	28.2	38	10.1	13.8	37
Leaf 5:						
1st leaflet	22.6	26.2	16	11.8	15.4	29

the plants, but may be connected in some way with water relations. Further analysis is in progress, as are also experiments with other plants.

Effects on flowering.—The production of an increased leaf area and the hastening of the appearance of the leaves might be expected to hasten the time of flowering. In the greenhouse the *Avena* plants from treated seeds flowered from three days to one

activity. The effect on time of flowering in *Avena* is perhaps an indirect one, resulting from the hastened growth.

It was shown in the first part of this paper that at the age of 7-8 days the roots of the treated plants are both longer and more numerous than those of the controls. Since the growth of a plant, especially in the early stages, is dependent on the amount of water

available, any increase in the amount of absorbing surface will cause an increase in rate. Plants with increased root systems will therefore grow ahead of the controls and will in general remain ahead. We have, in fact, observed that even when full-grown the root systems of the treated plants are larger than those of the controls (see table 7). Hence the hastened growth caused by auxin may very well be due to nothing more than the increased root system, though additional factors are, of course, not excluded.

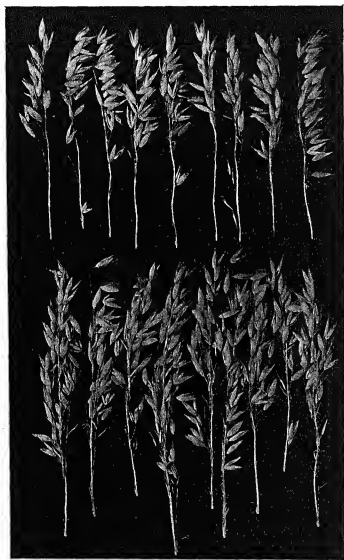


Fig. 5. Ears of the *Avena* plants of table 7. Top, control, no auxin; bottom, indole-acetic acid for the first 24 hours.

THE RELATION BETWEEN AUXIN AFTER-EFFECTS AND VERNALIZATION.—It was first shown by Gassner (1919) and later by numerous workers, especially in Russia (for literature see Bull. 17, Imperial Bureau of Plant Genetics, 1935), that if cereal seeds are allowed to enter the first stages of germination and are then stored at low temperature, on subsequent planting their flowering is considerably hastened. This process, known as vernalization or iarovisation, has been particularly successful with wheat; winter wheat after vernalization behaves like spring wheat in that it flowers in the year of planting. On the other hand, Blaauw and coworkers (see, for instance, Hartsema et al. 1930) have shown that the reverse is true for tulip bulbs; flowering in these is hastened by an

initial treatment with relatively high temperature. It will be obvious that the hastened growth which results from treatment of seed with auxin, as described above, suggests comparison with the phenomena of vernalization.

While this work was in progress two papers appeared by Cholodny (1936), embodying results somewhat similar to those reported here and also putting forward in brief a theory of vernalization based on auxin relations. However, the principal attempts at an analysis of vernalization made thus far seem to be those of Gregory and Purvis (1938), who have shown that vernalization may be reversed by drying down the seeds but that this treatment introduces an after-effect of high tiller production. The embryo of rye, isolated from the endosperm, may still be vernalized, so that whatever hormone supply is involved cannot be derived from the endosperm. In the earlier experiments of Purvis (1934) and Purvis and Gregory (1937) it is made clear that vernalization of winter rye involves two effects—(a) a hastening of spike growth and (b) a reduction in the minimal number of leaves which must be formed before the flower primordia are laid down.

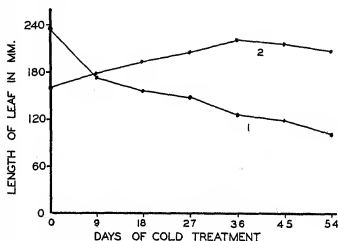


Fig. 6. Final lengths of the first leaves and lengths of the second leaves of refrigerated plants after 7 days in dark room. Experiment of Jan. 6–Mar. 3, 1937. Mean of 20 plants in each group.

In our experiments with long day plants (*Avena*), the flowering was hastened only slightly, and the main effect of the auxin treatment was evidently on the vegetative growth. Apparently the minimum number of leaves formed before flowering was not very different (both in *Avena* and *Lycopersicon*) in the treated and untreated. Although the auxin after-effect thus differs from vernalization, the following facts show that there is some relationship between the two phenomena.

In the first place, the earliest visible effect of vernalization in winter wheat consists of a shortening of the first leaf relative to that of controls. This is shown by figure 6, in which the final lengths of the first leaves of winter wheat are given for increasing periods of low temperature treatment.

In these experiments the seeds were soaked in water three hours at 25° and then kept at about 1°

for the periods shown (fig. 6). This was accomplished by starting a group of ten seeds every three days for fifty-one days. On the fifty-fourth day all were removed from the refrigerator and grown in water in the darkroom at 24°, in occasional red light, together with freshly started controls. It is clear that the final length of the first leaf, measured after its growth has ceased (seven days in dark room), decreases steadily with increasing cold treatment. The growth of the second leaf, however, is accelerated, as shown by the curve. The refrigerated plants showed, in fact, a marked acceleration of vegetative growth, particularly noticeable in the rate of emergence of this second leaf (table 10), but maintained also in subsequent leaves. A second experiment, with forty-one days of cold treatment, gave similar results.

TABLE 10. *Time of emergence of 2nd leaf in Winter Wheat kept at 1° and grown in the dark at 24°C. Experiment of January 7-March 9, 1937. Twenty plants in each group.*

Number of days of cold treatment	Number of plants showing 2nd leaf after six days
0	0
6	0
9-12	1
15-18	3
21-24	7
27-30	4
33-36	8
39-42	12
45-48	18
51-54	19

In this experiment a sample from each group of vernalized plants was grown in soil in the greenhouse in order to exclude the exhaustion of food as a factor in controlling leaf length. In figure 7 are shown three groups of the plants when nearly three weeks old; the decrease in length of the first leaf with increasing time of cold treatment is clearly seen, especially because, apart from this effect, the general growth increases with time of treatment.

It was shown above that soaking seeds of *Avena* in auxin (indole-acetic acid) does not inhibit the first leaf, but that even high auxin concentrations definitely increase it (see table 5). However, we have found that the inhibition of the first leaf may be imitated by auxin treatment, providing the treatment be delayed for twenty-four hours. Thus, if the seeds are soaked for twenty-four hours in water, then placed on filter paper for forty-eight hours in auxin solution (100 mg. per liter), a marked shortening of the first leaf results (table 11). The second leaf in most cases grows to its normal length, while the third and subsequent leaves are hastened in their development. These plants are therefore subjected to a transient inhibition, which is exerted mainly on the

first leaf. It is this transient inhibition which results also from vernalization.

In *Triticum*, unlike *Avena*, the first leaf is inhibited whether the auxin is applied to the seeds or to seedlings twenty-four hours after germination. An example of the effect of the delayed treatment is shown in table 11 for comparison with *Avena*, and a more complete experiment is summarized in figure 8. Here the plants were treated with auxin for twenty-four hours at different intervals after germination. The



Fig. 7. Plants refrigerated as figure 6 but planted in greenhouse. Photographed 18 days after end of refrigeration. Left to right; 12 days, 36 days, 54 days of cold treatment. Note the decreasing length of the first leaves.

treatment beginning when the plants are forty-eight hours old produces the maximum shortening of the first leaf. The final length of the second leaf is unchanged. The third and subsequent leaves are considerably accelerated in their growth, maximum acceleration resulting from the auxin treatment during the second twenty-four hours. The hastening of development is maintained, and by the emergence of the fifth leaf the plants treated during the second twenty-four hours were almost one full leaf ahead of the controls. In *Triticum*, therefore, auxin treatment at an early developmental stage produces marked shortening of the first leaf, and on the whole the greater this shortening the more the subsequent acceleration of the later leaves.

The effects of auxin treatment on the rate of growth of the leaves must be distinguished from its effects on the final size of the leaves. Early auxin treatment may increase considerably the final length of the leaves, as well as the rate of their development. On the other hand, at a certain stage in the embryonic development of the leaf, exposure of the plant to high auxin concentration may reduce the final size of the leaf, particularly of the first and second leaves, and yet also increase its rate of development.

It is of interest to note that Grace (1938) has recorded an increase in size of *Tropaeolum* leaves as a result of auxin treatment during growth.

Returning to vernalization, it may be suggested that, for the wheat here studied, the shortened first leaf, accompanied by a normal second leaf, probably provides a rapid diagnostic test for vernalization. Such a test would be of considerable value.

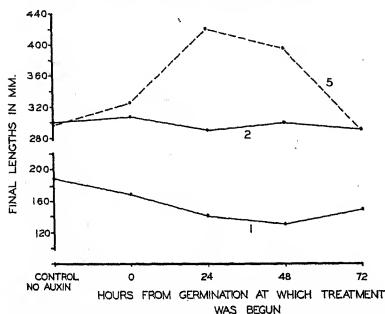


Fig. 8. Final lengths of the first and second leaves and lengths of fifth leaves at age 5 weeks. Plants treated with indole-acetic acid, 100 mg. per liter, for 24-hour periods only. Experiment of Jan. 10, 1938.

Finally, it is clear that in *Avena*, auxin treatment, under the right conditions of time and concentration, accelerates spike growth, enabling the treated plants to flower in some cases sooner and in no case later than the controls, in spite of producing one more leaf. If this effect is also obtainable with short day

treatment. The numerous after-effects of auxin treatment—namely, increase of percentage germination with low concentrations, improvement of the seedling root system, hastening of shoot growth and possibly increase in size of fruit with high concentrations—suggest that treatment of seed with auxins may prove valuable in a number of ways.

SUMMARY

In *Avena* and *Triticum* low auxin concentrations accelerate the rate of root elongation; high auxin concentrations inhibit it. As auxin, pure indole-3-acetic acid was used throughout.

If, however, the auxin is removed, the growth of the inhibited roots becomes faster than that of the controls, so that in a few days the treated roots are considerably longer.

In addition, treatment with high auxin concentrations increases the number of roots on the young plants.

After such treatment with high auxin concentrations, the general vegetative growth of the shoot is accelerated, the leaves may become both longer and wider, and the dry weight of the plants may be increased more than 50 per cent. There is a slight hastening of flowering, but this is probably due only to the accelerated vegetative growth. This general acceleration is considered to be an after-effect, due to the action of the auxin on the root system.

In *Avena*, if the auxin treatment is delayed until the plants are twenty-four hours old or more, the length of the first leaf is decreased. In *Triticum*, the length of the first leaf is decreased both by this de-

TABLE 11. Effect of delayed auxin treatment on leaf length. Plants in dark room during treatment.

Plant	Age at which auxin was applied	Grown in	Final lengths of leaves	
			First	Second
<i>Avena</i>	Control; no auxin	Greenhouse	143	217
	24th to 72nd hour	Greenhouse	110	213
<i>Triticum</i>	Control; no auxin	Dark room	244	206
	24th to 72nd hour	Dark room	119	188

plants, then auxin treatment resembles vernalization to this extent. Hence, it is suggested that one result of vernalization is to give the young plant a prolonged treatment with its own auxin. This would account for the phenomena which are common to the auxin treatment and vernalization. The additional phenomena which are different in the two treatments are essentially that vernalization reduces the minimal number of leaves without markedly affecting the general vegetative growth, while auxin treatment, with no decrease or a small increase in the minimal number of leaves, increases the general vegetative develop-

ment. The growth of the subsequent leaves is nevertheless hastened. The same result—i.e., shortening of the first leaf, followed by general acceleration of vegetative growth—is obtained in *Triticum* by low temperature storage of the wetted seed—that is, vernalization. It is therefore tentatively suggested that the vegetative effects of vernalization are due to the prolonged exposure of the seed to its internal auxin supply.

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THIAMIN (VITAMIN B₁) AND THE GROWTH OF ROOTS: THE RELATION OF CHEMICAL STRUCTURE TO PHYSIOLOGICAL ACTIVITY¹

James Bonner

IN EARLIER papers it has been shown that thiamin² (vitamin B₁) is essential to the growth of the pea root (Bonner, 1937; Bonner and Addicott, 1937). That this substance may be regarded as a general root growth factor is indicated by the fact that it also exerts a great effect upon the roots of tomato (Robbins and Bartley, 1937a, 1937b), of *Citrus* and of *Camellia* (Went, Bonner, and Warner, 1938), as well as upon the roots of many other plants (Warner, unpublished). In the normal plant thiamin is probably supplied by the green leaves; in the seed-

ling, by the seed itself; thiamin is hence to be regarded as a typical phytohormone or carrier of a growth correlation. If, however, the tip of the root is excised and grown in vitro, thiamin must be supplied to the nutrient medium if continued growth is to occur. In order that we may better understand the manner in which thiamin functions as a root growth factor, it is essential that we know which portions of the thiamin molecule are necessary and which are not—in other words, that we know which the "active groups" of the molecule are. This may be determined by a study of the root growth activity of thiamin analogs, substances closely related to the vitamin and differing from it only slightly in the nature or position of one or more chemical groups.

METHODS.—Pea roots cultured in vitro were used as the test material in the present investigation. Pea seeds of the variety "Perfection"³ were washed in alcohol, sterilized for 20 minutes in 0.1 per cent HgCl₂, washed with sterile water, and allowed to germinate for 48 hours in sterile Petri dishes at 25°C.

³Obtained through the courtesy of the Ferry-Morse Seed Company, San Francisco.

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² In this and subsequent papers concerning the role of vitamin B₁ in plant growth, this substance will be referred to as "thiamin" in accordance with the nomenclature adopted by chemists in this country. In view of the general occurrence and function of vitamin B₁ the name "thiamin" which suggests the chemical nature of the molecule, seems also somewhat more appropriate than the older name "aneurin" which suggests rather a particular function of the substance in the higher animals.

At the end of this time, root tips approximately 3 mm. long were excised from the seedling roots and placed in Petri dishes containing 15 cc. of "pea root medium" having the composition shown in table 1. The tips as excised from the seed contain considerable amounts of thiamin, and, as noted in an earlier publication (Bonner and Addicott, 1937), are able to grow 60 mm. or more during their first week of culture in vitro. At the end of this first week, tips 1 cm. long were again removed. These tips contain much less thiamin than do the original tips, and hence grow much less—i.e. approximately 10 mm.—if they are transferred once more to a thiamin-free medium. If this process of subculture is repeated for a third time, the roots do not grow at all. Correspondingly, they contain no detectable amount of thiamin. These relations between thiamin content of the excised root tip and its subsequent growth are summarized in figure 1.⁴

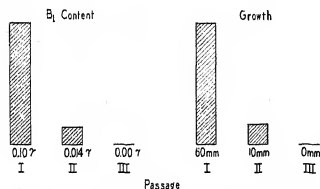


Fig. 1. The relation between thiamin (vitamin B₁) content and subsequent growth of pea root tips, in vitro. I refers to tips as excised from the germinating seed, II refers to roots which have grown for one week in vitro, and III refers to roots which have grown for two weeks in vitro.

Roots which have been kept in thiamin-free medium until their own store of B₁ is exhausted are not capable of subsequent growth. This is apparently because cell divisions in the meristem cease almost completely (Addicott, 1937). At the end of the third passage in B₁ free medium there is practically no meristematic region left, and the roots consist only of large cells and well differentiated tissues. It is therefore not possible to use roots which are completely free of thiamin for the assay of vitamin B₁ activity. Tips from roots which have been only one passage in vitro, however, are well suited to this purpose. If such roots are cultured for the second passage in thiamin-free medium, they grow in general approximately 10 mm. If, however, they are cultured into medium containing thiamin, they may grow as much as 60–100 mm. Roots in this second passage have accordingly been used for all of the work described below. Tips 1 cm. long were excised from roots which had already been 7 days in culture. These tips were placed in culture solution of the

⁴The thiamin determinations were done by James Erickson using the *Phycomyces* test. Details of this work will appear elsewhere.

composition shown in table 1 to which had been added the desired amount of vitamin B₁ or its analogs.⁵ These tips were allowed to grow at room temperature for 7 days and were then measured. Experiments were run with sets of 200 roots, divided into 10 sets. One set of 20 roots was reserved in each experiment for determination of the growth in thiamin-free medium. Each of the other 9 sets of 20 roots each was used for the determination of the root growth activity of one substance or mixture of substances. Each experiment was repeated one or more times, so that the determination of the root growth activity for any one of the substances listed below is based upon measurements of 40 to 200 or more roots for each concentration.

TABLE 1. The composition of pea root medium.

Ca(NO ₃) ₂ · 4H ₂ O	236 mgs.
MgSO ₄ · 7H ₂ O	26 "
KNO ₃	81 "
KCl	65 "
KH ₂ PO ₄	12 "
Ferric tartrate	1.5 "
Sucrose	40 gms.
Redistilled water	1 liter.

EXPERIMENTS WITH THE TWO PORTIONS OF THE VITAMIN MOLECULE.—The structure of the thiamin molecule is reproduced in figure 2 (Williams, 1936). It may be seen that the molecule consists essentially of a substituted pyrimidine ring linked through a methylene bridge to a substituted thiazole nucleus. The final step of the Williams synthesis of the vitamin in vitro is the condensation of the suitably substituted bromo-methyl pyrimidine with the suitably substituted thiazole. Each of these substances is also shown in figure 2. The obvious test is therefore to

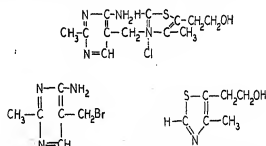


Fig. 2. The structure of thiamin (above), of the "vitamin pyrimidine" (left), and of the "vitamin thiazole" (right).

determine whether or not the organism itself is able to accomplish this synthesis. Experiments of this nature have been reported by Schopfer and Jung (1937), Sinclair (1937), Robbins and Kavanagh (1937a, 1937b), and Knight (1937), among others.⁶ In these cases it has been found that mixtures of the appropriate pyrimidine and thiazole are able to replace the vitamin itself

⁵Culture solutions were sterilized in all cases by autoclaving at 15 pounds pressure for 25 minutes.

⁶The 5 amino methyl pyrimidine or the 5 ethoxy methyl pyrimidine as well as the 5 bromo methyl pyrimidine have been used by these workers.

for some lower plants such as *Phycomyces* and *Staphylococcus*. In table 2 it may be seen that an equimolar solution of the pyrimidine and the thiazole is just as effective as is thiamin itself in promoting the growth of pea roots. Either the pyrimidine portion of the thiazole portion alone, however, is quite without effect, even in the highest concentrations. The behavior of pea roots is thus in contrast with that of tomato roots as reported by Robbins and Bartley (1937b), who found that thiazole alone could replace thiamin, at least to some extent. We may also conclude from table 2 that pea roots are unable to synthesize either the thiazole portion or the pyrimidine portion from the sucrose and inorganic materials of the culture medium.

TABLE 2. Comparison of the effects of thiamin (vitamin B₁) and of an equimolar solution of pyrimidine and thiazole as growth factors for pea roots.

Growth factor	Molal concn.	Growth in mm. per week over control			
		10 ⁻⁵	10 ⁻⁷	10 ⁻⁹	10 ⁻¹¹
Vitamin B ₁	40	40	34	10	
Pyrimidine + thiazole	40	43	36	14	
Pyrimidine alone	0	0	0	0	
Thiazole alone	0	0	0	0	

One might next enquire as to whether or not the roots actually do synthesize thiamin when they are supplied with the two portions of the molecule. Several experiments were therefore carried out in which the ratio of pyrimidine to thiazole was widely varied. The results of a typical experiment are presented in table 3. It may be seen at once that the component which is present in the smaller amount limits the total growth activity. A concentration of 10⁻⁹ molal thia-

(1937a, 1937b) for *Phycomyces*, and they suggest strongly that the two portions of the vitamin molecule are used by the organism in equimolecular amounts, as they would be if the vitamin synthesis were actually carried out.

THE ACTIVITIES OF THIAZOLE ANALOGS.—Chemical analogs of the vitamin thiazole were obtained through the courtesy of Dr. E. R. Buchman and Dr. E. M. Richardson.⁷ None of these thiazoles possessed any activity upon pea roots if tested in the absence of an appropriate pyrimidine. If, however, they were tested in the presence of an excess of the 5-bromo-methyl pyrimidine (fig. 2), they exhibited widely varying effects upon growth which are summarized in table 4. The "relative growth effects" in this table are given for a concentration of 10⁻⁷ molal. This concentration of the vitamin thiazole (in the presence of the pyrimidine) causes a maximum growth—i.e., still higher concentrations do not further increase the growth. A "relative effect on growth" of 50 per cent in table 4 indicates, then, that at a concentration of 10⁻⁷ molal the substance in question causes a pea root growth response which is 50 per cent of that caused by the vitamin thiazole in this concentration.

The first substance in table 4 is the vitamin thiazole itself. The second is an analog in which the OH group of the former substance has been replaced by a Cl atom. This can be hydrolyzed in vitro under certain conditions to the vitamin thiazole. This, together with the fact that its activity⁸ is identical with that of the vitamin thiazole, suggests that it may be hydrolyzed in vivo to the latter, or that in any case it is readily metabolized in the same fashion as the vitamin. Substances 3 to 8 are closely related to the vitamin thiazole but are all inactive upon pea roots. It is to be noted that they have in common the absence of an OH group from the molecule.

TABLE 3. Influence of the ratio of pyrimidine to thiazole upon the growth of pea roots.

Molal concentration of varied component	Growth: mm. per week over control: (Varied component of medium)		
	Pyr. + Th.	Th. (Pyr. = 10 ⁻⁹ M)	Pyr. (Th. = 10 ⁻⁹ M)
10 ⁻⁷	53 ± 2	33 ± 2	28 ± 2
10 ⁻⁹	29 ± 2	29 ± 2	29 ± 2
10 ⁻¹¹	9 ± 2	13 ± 1	12 ± 1

zole gives essentially the same amount of root growth with an equal concentration of pyrimidine or with 100 times this concentration. If, on the other hand, both components are increased by 100 times, root growth is correspondingly increased. Smaller differences between the concentrations of pyrimidine and thiazole gave the same, although less striking, result, due to the fact, as shown in table 2, that root growth increases but slowly with increase in concentration of the growth factor. These results, however, are in agreement with those of Robbins and Kavanagh

Substances 9 to 11 are isomeric with the vitamin thiazole and differ from it only in the position of the substituent OH group. In the case of substance 11 a Cl atom rather than an OH group is actually present, but as shown by substance 2, this does not

⁷ The synthesis of these analogs was made possible by a grant from the Research Corporation.

⁸ The activity of this and other of the thiazole analogs reported on here cannot be due to contamination with significant amounts of the vitamin thiazole. Evidence in support of this will be presented elsewhere.

TABLE 4. *The relation of chemical structure of the thiazole nucleus to its activity as a growth factor for pea roots. (All tested in the presence of the appropriate pyrimidine.)*

$$\begin{array}{c}
 \text{S} \quad \text{C}-\text{R}_3 \\
 \diagup \quad \diagdown \\
 \text{R}_2-\text{C} \quad \text{C}-\text{R}_4 \\
 \diagdown \quad \diagup \\
 \text{N}
 \end{array}$$

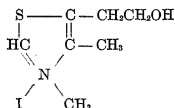
Number	R ₂	R ₄	R ₃	Relative effect on growth; %
1 ¹	-CH ₂ CH ₂ OH	-CH ₃	H	100
2	-CH ₂ CH ₂ Cl	-CH ₃	-H	100
3	-CH ₂ CH ₃	-CH ₃	H	0
4	-COCH ₃	-CH ₃	H	0
5	-CH ₂ COOH	-CH ₃	H	0
6	-CHO	-CH ₃	H	0
7	H	-CH ₃	H	0
8	-CH ₂ CH ₂ N(CH ₃) ₃ Br	-CH ₃	H	0
9	-CHOHCH ₃	-CH ₃	H	30
10	-CH ₂ CH ₃	-CH ₂ OH	H	100
11 ²	-CH ₃	-CH ₂ CH ₂ Cl	H	75
12	-CH ₂ CH ₂ OH	-CH ₃	-CH ₃	35
13	-(CH ₂) ₃ OH	-CH ₃	H	75
14	-CH ₂ CHOHCH ₃	-CH ₃	H	100
15 ³	-CH ₂ Br	-CH ₃	H	90
16	-CH=CH ₂	-CH ₃	H	100
17	-CH ₂ CH ₂ OH	-CH ₃	-NH ₂	0
18 ⁴	-methiodide of 1.			0

¹ This is the vitamin thiazole.

² Preliminary result.

³ This substance polymerizes rapidly with loss of activity.

⁴ Quaternary salt of structure:



affect root growth activity. All of these three isomers are active upon pea roots. The OH group may be changed from the beta to the alpha position of the 5 ethyl side chain. It may be even placed in the 4 position, upon either a methyl or an ethyl side chain.

Substances 12, 13, and 14 are three isomers containing one more CH₂ group than does the vitamin thiazole. All are active as growth factors for pea roots. Substance 15 is similarly a compound having one less CH₂ group than does the vitamin thiazole and is also active upon pea roots. In 15, also, a halogen atom rather than an OH group is present, but here again it may readily be hydrolyzed *in vivo* to an OH group.

Substance 16 contains a vinyl side chain in the 5 position. It has been shown (number 3) that the corresponding saturated compound is inactive. It would seem reasonable to suppose that the activity of the vinyl compound is due to the possibility for addition at the double bond. If a molecule of water

were added at this double bond, either number 1 (the vitamin thiazole itself) or number 9 would result. Both of these are active. It is possible, however, that an addition other than that of water may take place at this double bond, for example, of pyrophosphoric acid, resulting then in the direct formation of co-carboxylase (see below).

In substance 17 an NH₂ group is substituted in the 2 position. This substance is inactive, in contrast thus with substance 11 in which a CH₃ is substituted in the same position.

Number 18 is the methiodide of the vitamin thiazole, and it is of interest to note that this substance is inactive. The thiazole is present in the vitamin molecule as a quaternary salt. This by itself, however, is not sufficient. The pyrimidine portion must also play a specific role, a question which is taken up more fully below. This methiodide is inactive not only alone, but also in mixtures with the 5-bromo-methyl or with the 5-amino-methyl pyrimidine, combinations similar to those for which Schopfer (1937)

TABLE 5. Tests for the dissociation of thiazole quaternary salts. Dissociation is measured by amount of recombination with the vitamin pyrimidine and consequent activity of the mixture as a growth factor for pea roots.

Mixture	Activity
Methiodide of vitamin thiazole	0
Same + vitamin pyrimidine	0
2-chloro iso-aneurin iodide (Todd)	0
Same + vitamin pyrimidine	0
Thiochrome, alone	0
Thiochrome + vitamin pyrimidine	0
Thiochrome + vitamin thiazole	0

has reported activity for *Phycomyces*. This lack of activity in the presence of the pyrimidine indicates that no appreciable dissociation of the quaternary salt to give the free thiazole takes place. Another quaternary salt was tested both alone and in the presence of the appropriate pyrimidine, and the results, as shown in table 5, indicate that for it also no significant dissociation occurs. Thiochrome, a closely re-

The substances 1 to 4 are pyrimidines which differ in the nature of the substituent upon the 5 methyl group. In these four substances the substituent group is relatively reactive, and hence the pyrimidine can presumably link readily with the thiazole. These four substances are all otherwise similar to the vitamin pyrimidine and they are all active in supporting the growth of pea roots. If, however, the substituent upon the 5 methyl group is relatively unreactive, as in the compounds 6 and 7, the pyrimidine is found to be inactive. The presence of such a reactive group is then a prerequisite for activity as a "vitamin pyrimidine." It should be stressed that to test other pyrimidines in which this condition is not fulfilled would be meaningless since it sheds no further light upon the structural requirements. Accordingly, such analogs are not included in table 6, although a considerable number of them were tested and found, as expected, to be inactive.

An OH group is unable to replace the amino group in the 6 position, as is shown by the inactivity of substances 8 to 10, all of which possess reactive groups in position 5. Substance 12 is 2-chloro-isoaneurin iodide and, as indicated in table 6, is already coupled

TABLE 6. The relation of chemical structure of the pyrimidine nucleus to its activity as a growth factor for pea roots. (All tested in the presence of the appropriate thiazole.)

Number	$ \begin{array}{c} \text{N} - \text{C} - \text{R}_2 \\ \parallel \quad \parallel \\ \text{R}_2 - \text{C} \quad \text{C} - \text{R}_3 \\ \parallel \quad \parallel \\ \text{N} = \text{C} - \text{R}_4 \end{array} $				Relative effect on growth; %
	R ₂	R ₄	R ₃	R ₅	
1 ¹	-CH ₃	H	-CH ₂ Br	-NH ₂	100
2	-CH ₃	H	-CH ₂ NH ₂	-NH ₂	95
3	-CH ₃	H	-CH ₂ OEt	-NH ₂	25
4	-CH ₃	H	-CH ₂ NHC ₂ H ₅	-NH ₂	100
5	-CH ₃	H	H	-NH ₂	0
6	-CH ₃	H	-CH ₂ COOH	-NH ₂	0
7	-CH ₃	H	-CH ₂ CONH ₂	-NH ₂	0
8	-CH ₃	H	-CH ₂ OEt	-OH	0
9	-CH ₃	H	-CH ₂ NH ₂	-OH	0
10	-CH ₃	H	-CH ₂ OH	-OH	0
11	-OH	-CH ₃	-CH ₂ OH	-OH	0
12 ²	Cl	-CH ₃	-CH ₂ -thiazole	-NH ₂	0

¹ This is the vitamin pyrimidine.

² 2-chloro, iso-aneurin iodide (Todd).

lated oxidation product of the vitamin was found to be inactive both alone and in combination with either the pyrimidine or thiazole.

ACTIVITIES OF PYRIMIDINE ANALOGS.—Analogues of the vitamin pyrimidine were obtained through the courtesy of Dr. A. R. Todd (Lister Institute), Dr. J. K. Cline (Merck and Co., Inc.), and Dr. E. R. Buchman (California Institute of Technology) and were tested in the presence of the vitamin thiazole (fig. 2). The activities of these analogs are summarized in table 6.

with the vitamin thiazole. This substance is inactive, as was shown in another connection in table 5.

DISCUSSION.—It is now generally recognized that thiamin (vitamin B₁) is an essential growth factor for higher as well as for lower plants. Studies of the relation of the structure of the thiamin molecule to its physiological activity, however, have been made in relatively few cases, notably for *Phycomyces blakesleeana* (Schopfer, 1937; Robbins and Kavanagh, 1937, 1938a, 1938b; Sinclair, 1937) and for

Staphylococcus aureus (Knight, 1937). Comparisons of the requirements of the pea root with those of other organisms must therefore be limited in scope. Robbins and Bartley (1937a, 1937b) have reported experiments upon the tomato root which may be interpreted as signifying that the tomato root is able to cover at least a portion of its pyrimidine requirements by synthesis, so that the thiazole portion of the thiamin molecule is able to act alone as a growth factor. The tomato root then would resemble the fungus *Mucor Ramannianus* (Müller and Schopper, 1937) in its capacity for synthesis of the vitamin pyrimidine. The pea root is unable to carry out this synthesis and requires that both the pyrimidine and the thiazole portions be supplied to it if growth is to take place. The pea root resembles in this respect both *Phycomyces* and *Staphylococcus*, rather than the tomato root. The two portions of the vitamin may be supplied either linked together, as in the vitamin, or they may be supplied as a mixture of the two substances. In this case, however, it seems very probable from the evidence of table 3 that an in vivo linking of the two portions takes place.

The pyrimidine portion of the molecule is essential for vitamin activity quite aside from the fact that it forms a quaternary salt with the thiazole portion. Thus, simple quaternary salts of the vitamin thiazole—e.g., the methiodide—are inactive in supporting root growth. A specificity of the pyrimidine portion is further indicated by the essential nature of the 6 amino group.

It is in its wide latitude of active thiazole structures that the pea root differs most markedly in its requirements from those of *Phycomyces*. For the pea root (with the exception of the 2 amino vitamin thiazole, compound 17 in table 4) it is only necessary that an OH group (or a group readily metabolizable to an OH group) be present in the thiazole molecule. This OH group may be in any one of a number of positions in the molecule. *Phycomyces*, on the other hand, requires that the OH group be present essentially as in the vitamin molecule, as will be shown

in a later paper. That in both cases an OH group should be essential does not occasion surprise in view of the finding of Lohman and Schuster (1937) that thiamin, for its function as co-carboxylase, must be esterified with pyro-phosphoric acid.

The pea root because of its relatively low specificity would seem to offer a peculiarly favorable object for the study of the relation of chemical structure to the physiological activity of vitamin B₁. A further investigation of the physiology of vitamin B₁ in root growth is under way.

SUMMARY

The role of thiamin (vitamin B₁) in the growth of excised pea roots in vitro has been further investigated. Root tips, as excised from the seedling plant, contain a considerable "reserve" of thiamin. For this reason thiamin does not limit root growth in vitro until the roots have grown 60 or more mm. and tips of these roots have been transferred to fresh medium.

The pea root is able to utilize an equimolar solution of the pyrimidine and thiazole portions of the thiamin molecule. It is probable that these two portions are synthesized in vivo to the vitamin molecule itself.

The pea root can utilize, as the thiazole component of such a mixture, a considerable number of substituted thiazole compounds. It is essential, however, that an hydroxyl group (or group readily metabolizable to such) be present in the molecule if the substance is to possess activity as a root growth factor. Quaternary salts, unless the substituent possesses the prerequisites of an active pyrimidine, cannot be utilized.

As the pyrimidine constituent the root can utilize 2,5 dimethyl, 6 amino pyrimidines, substituted in the 5 methyl position by groups which will permit of the formation of quaternary salts. The 6 amino group cannot be replaced by a hydroxyl group.

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THE NUTRITION OF THE SPOROPHYTE IN THE HEPATICAE¹

Harold C. Bold

THE HEPATICS have always occupied a place of prominence in discussions of such problems as the origin of a land flora and of the nature, relationship, and origin of the alternating generations so universally observed. There are few who would dispute the premise that at least certain of our extant hepatics are not far removed from the pioneer terrestrial plants which fortuitously or orthogenetically departed from their submerged habitat, thus initiating a land flora. In connection with the second problem, that of alternation of generations, the Hepaticae have been intensively and comparatively investigated in an attempt to obtain a clue to the solution of this difficult problem; as a result of these studies, though the series be read as one of reduction or one of advancing development, all investigators are apparently in accord that it is only in the Anthocerotales (if these be credited with but ordinal rank) that the sporophyte has achieved some degree of autotrophism by photosynthesis. There are, however, occasional reports in the literature which contradict this conclusion. Mirbel (1835), for example, described and figured the young sporophytes, elaters, and what were probably spore mother cells of *Marchantia polymorpha* as being rich in chlorophyll content. Hofmeister (1862) states that the immature sporophyte of *Jungermannia divaricata* contains chlorophyll. Leitgeb (1879), Campbell (1928), and Goebel (1930) all assert that chlorophyll is present in the developing sporophytes of *Sphaerocarpos*; the last author goes so far as to state that since the stalk of the sporophyte breaks down early in ontogeny, this structure is dependent nutritionally only on itself. He reports similarly the presence of chlorophyll in the sporophyte of *Aneura*. Motte (1932) states that the sporophyte of *Pellia Fabbriana* contains chloroplasts; in the same compilation Garjeanne (1932) reports that the sporophytes of liverworts other than *Anthoceros* contain chlorophyll only in the earlier phases of their development, but he gives no figures or authority for such a statement. Recently Patterson (1933) mentions the occurrence of a "few small green chromatophores" in the seta of *Dumortiera hirsuta*. In spite of these reports the sporophytes of the Marchantiales, Jungermanniales, and Sphaerocarpaceae are usually regarded as lacking chlorophyll and with it the capacity for self nutrition; in respect to their relation to the gametophyte which bears them they are described by such terms as "parasitic" and "dependent." This viewpoint is well illustrated by the following quotation from a standard elementary text (Holman and Robbins, 1938) which in describing the sporo-

phyte of *Anthoceros* says: "In all the liverworts thus far discussed and in fact in most liverworts, the sporophyte is parasitic upon the gametophyte, securing from it all its water and food. In striking contrast with these forms are the genus *Anthoceros* and two other closely related genera in which the sporophytes are green and able to carry on photosynthesis and thus to make their own food although they remain attached to the gametophyte and are dependent upon it for their supply of water and salts from the soil." Nor is this doctrine to be found alone in elementary treatises. Bower (1935), for example, states in speaking of the Anthocerotales: "In particular the sporogonium of the remaining liverworts is habitually colorless, and specialized as a spore-bearing and distributing body; and photosynthesis is carried on only by the gametophyte." In his recent text Smith (1938) expresses a similar conclusion. Further multiplicity of quotation seems irrelevant since this doctrine is so widely taught and accepted.

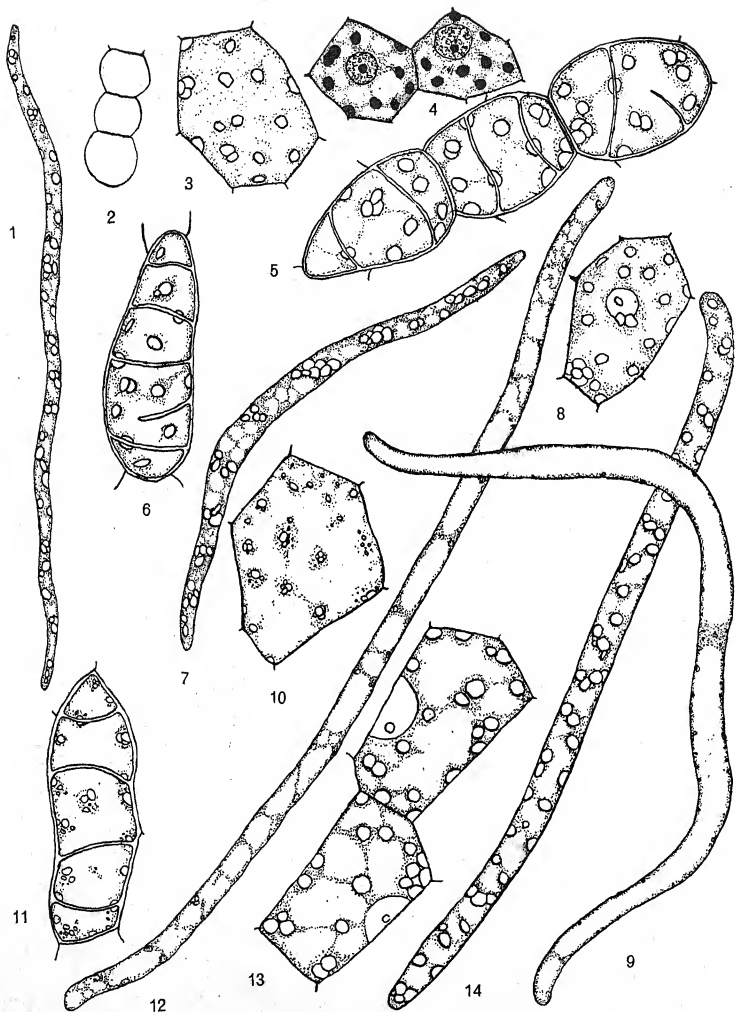
MATERIAL AND METHODS.—Several years ago, in attempting to ascertain before fixation whether or not the sporophytes of a certain collection of *Dumortiera hirsuta* had passed beyond the stage of sporogenesis, I chanced to section some of the living archegoniophores with their developing sporophytes. At the time it was noted that in gross aspect the sectioned sporophytes were green, but the significance of this did not impress me especially until the same result was experienced in sectioning the living sporophytes of *Mannia rupestris*² during the early part of this year. This led me to inquire into the question of the presence of chlorophyll in the hepatican sporophyte, and during the past months I have made an attempt to examine as many fruiting plants as possible. The following material was studied: *Ricciocarpus natans*, *Marchantia polymorpha*, *Asterella tenella*, *Dumortiera hirsuta*, *Mannia rupestris*, *Pellia epiphylla*, *Frullania* sp., *Cephalozia* sp., and *Blepharostoma trichophyllum*. In all cases the sporophytes were examined macroscopically and microscopically for the presence of chlorophyll.

OBSERVATIONS.—Since *Marchantia polymorpha* was abundant in the University greenhouse, with sporophytes in various stages of development, it was possible to make a more complete study of it than of any other species. The eggs themselves seem to be colorless both before and after fertilization, although they probably contain plastid primordia, as Davis (1899) and Scherrer (1913) have reported for *Anthoceros*. In the classical "Botanische Wandtafeln" of Kny (LXXXVII, 1) the group of archegonia of *Marchantia* figured in position in the gametophore have

² The organism here called *Mannia rupestris* was provisionally so identified by Professor A. W. Evans of Yale University.

¹ Received for publication May 23, 1938.

The writer wishes to take this opportunity to express his gratitude and appreciation to Professor C. E. Allen for criticisms and for reading the manuscript.



All figures were drawn with the aid of a Zeiss camera lucida at an approximate magnification of 666 \times and have been reproduced without reduction.

Fig. 1-14. Fig. 1-6. *Marchantia polymorpha*.—Fig. 1. Immature elater.—Fig. 2. Outline drawing of spore mother cells from the same capsule.—Fig. 3. Seta cell.—Fig. 4. Foot cell from stained section.—Fig. 5, 6. Capsule wall cells.—Fig. 7. *Mannia rupestris*. Immature elater.—Fig. 8, 9. *Reboulia hemisphaerica*.—Fig. 8. Seta cell.—Fig. 9. Elater.—Fig. 10-12. *Dumortiera hirsuta*.—Fig. 10. Seta cell.—Fig. 11. Capsule wall cell.—Fig. 12. Elater.—Fig. 13, 14. *Asterella tenella*.—Fig. 13. Seta cells.—Fig. 14. Young elater.

their eggs colored a pale green; but this may be interpreted as due to the reflection from the chlorophylliferous venter cells beneath, since the detailed drawing of a single archegonium on the same chart (LXXXVII, 11) shows the egg with colorless cytoplasm. In the living condition the few-celled embryo and later spherical stages are likewise colorless, but by the time differentiation into foot, seta, and capsule regions has occurred, the entire sporophyte is a deep, dull green in color. The Kny charts show this clearly in their illustration of this stage (LXXXVIII, 6). Mirbel's (1835) colored illustrations likewise indicate that the sporophyte is chlorophylliferous. The sporophyte retains its green appearance until the spores are developed and the capsule wall and elaters have developed their secondary thickenings. It is impossible to obtain sufficiently clear views of the living sporophyte cells to trace the exact origin of the chloroplasts. I have illustrated in figures 1 to 6 certain cells from an immature sporophyte in which the sporogenous tissue was in the spore mother cell stage. Figure 1 represents a young elater which contains numerous chloroplasts, each of which in turn contains one or several minute starch grains. Mirbel (1835) clearly figures the young elaters filled with chloroplasts. In my material the sporocytes were colorless, while Mirbel apparently found them to be green. I am at a loss to explain this discrepancy. Figures 3 and 4 illustrate respectively a cell from the unelongated seta and one from the foot. The latter is drawn from a stained section, but both types of cells in the living sporophyte contained numerous and active chloroplasts. The same condition obtains in the wall cells of the capsule, several of which are shown in figures 5 and 6. The chloroplasts break up into fragments as the time of capsule dehiscence approaches, but the cytoplasm as a whole retains a green cast for some time after the plastids have lost their individuality. A study of developing sporophytes stained with iodine at various stages indicates that the rate of photosynthesis does not exceed that at which the photosynthate is utilized until after sporogenesis is completed, for it is only after this that any considerable amount of starch occurs in the cells in the form of large storage grains.

While it was impossible to study the sporophytes of the remaining genera in as many stages of ontogeny as those of *Marchantia*, my observations convince me that all of them, except possibly *Ricciocarpus natans*, are photosynthetic and therefore autotrophic to a considerable degree, as evidenced by the presence of chlorophyll. The presence of starch grains alone cannot, of course, be construed as unequivocal evidence of autotrophism, since these may arise from soluble carbohydrates which have diffused into the sporophyte from a gametophyte source. It seems to the writer, however, that the occurrence of minute starch grains within the chloroplasts themselves indicates that the latter are actively functioning.

Figure 7 illustrates an immature elater of *Mannia rupestris* in whose vacuolate cytoplasm occur groups

of chloroplasts each with included starch grains. Figures 8 and 9 represent respectively a seta cell and elater of *Reboulia*. The plastids in the latter have already begun to disintegrate and are scattered throughout the peripheral cytoplasm in the form of rather minute droplets of pigment. In figures 10, 11, and 12 are shown a seta cell, capsule wall cell, and elater of *Dumortiera hirsuta*. In the material available these cells contained abundant starch grains surrounded by clusters of small chloroplast fragments or chlorophyll droplets, and I am unwilling to state categorically whether or not an earlier stage may not have been similar to *Marchantia* in the presence of large, unfragmented chloroplasts. The presence of chlorophyll, however, in the sporophytes of *Dumortiera* cannot be questioned. The chloroplasts in the capsule wall cells and elaters of *Asterella tenella* (fig. 13, 14) are slightly larger than those of *Marchantia* and, like the latter, contain one or several starch grains within them. Studies of the sporophyte of *Ricciocarpus natans* are at present incomplete for lack of material containing sporophytes in the earlier stages of development. As pointed out by Garber (1904) and others, the single layer of wall cells in the sporophyte of *R. natans* breaks down early, and in the available material I have been unable to obtain clear and convincing views of these cells. The sporocytes themselves are apparently devoid of chlorophyll throughout their ontogeny. In connection with these observations Goebel's (1930) report of chlorophylliferous "sterile" cells in the capsule of *Corsinia Marchantioides* and of inhibited elaters in *Mono-selenium tenerum* is possibly significant.

The Jungermanniales are usually divided into two families, the Metzgeriaceae and the Jungermanniaceae. Among the former I have had favorable fruiting material only of *Pellia epiphylla* (fig. 15-18). In the smallest sporophytes available the seta had already elongated sufficiently to raise the capsule above the margin of the calyptra. The short pre-elongated seta and young capsule are clearly green in appearance macroscopically; the color of the former becomes paler and finally white by the time it has elongated to a length of 22 mm. I have drawn living cells from a 3 mm., 6 mm., and 22 mm. seta in optical sections as figures 15, 16, and 18, respectively. Microscopically the whole immature sporophyte presents a dense green appearance, and examination of the cells of the shortest seta available (fig. 15) revealed the presence of numerous starch grains surrounded by minute green droplets or fragments of chloroplasts. The cytoplasm is dense and occupies most of the lumen of the cell, although some vacuolization has already begun. In successively older stages (fig. 16, 18) the gross changes in the seta may be inferred from comparative examination of its component cells; rapid elongation of the cell occurs, and the cytoplasm is displaced by the coalescence of droplets of liquid to an entirely peripheral position, a process no different from that observable in the elongation of the axes of the higher plants. The starch grains dis-

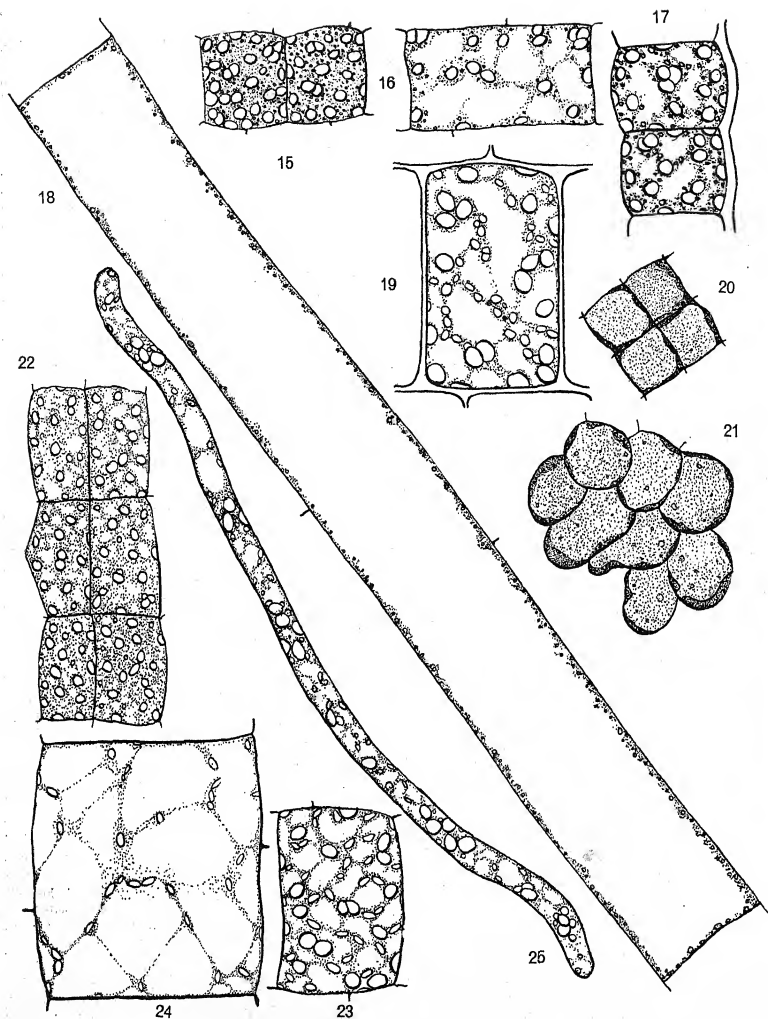


Fig. 15-25. Fig. 15, 16, 18. *Pellia epiphylla*. Cells from successively more elongated setae.—Fig. 17. Capsule wall cell.—Fig. 19. *Blepharostoma trichophyllum*. Seta cell.—Fig. 20. *Frullania* sp. Seta cells.—Fig. 21. Foot cells.—Fig. 22-25. *Cephalozia* sp. Cells from successively older setae.

appear in the most elongated seta cells, and the few remaining chlorophyll droplets are scattered in the cytoplasm. It is probable that the starch is digested and utilized in augmenting the cell walls of the rapidly elongating seta cells. Figure 17 illustrates two of the outermost cells of the capsule wall, and in these abundant starch grains and minute chloroplasts are also visible.

Among the Jungermanniaceae studied the sporophytes are also strongly chlorophylliferous. Figure 19 represents a cell from the pre-elongated seta of *Blepharostoma trichophyllum*. The cell walls are thick and probably somewhat gelatinous, and the cytoplasm contains numerous large starch grains as well as smaller chloroplasts. The latter are significant perhaps, since they are intermediate in size between the large type found in *Marchantia* and *Asterella* and the minute ones found in *Pellia* and *Dumortiera*. Of course the ontogeny of the plastids in the latter has not been sufficiently completely followed to preclude their origin by fragmentation of larger bodies. The youngest sporophytes I have been able to study among the Jungermanniaceae were found in a species of *Frullania* (fig. 20, 21). The plastids in this species are clearly present in the cells of the haustorial foot (fig. 21) as well as in the capsule and seta cells. In the material available, however, they were somewhat indefinite in extent, appearing more like local aggregations of cytoplasm impregnated with chlorophyll, and were pale green in color. They are not unlike the parietal chloroplasts of certain algae, and had later stages been available, it would have been interesting to study the plastid condition in the more mature sporophyte. Finally figures 22 through 25 were taken from the setae of sporophytes of a species of *Cephalozia*. The relation in size between plastid and starch grain is similar here to that which obtains in the genus *Blepharostoma*. Changes similar to those observed in the cells of *Pellia* occur in the elongation of the seta of *Cephalozia* (fig. 23, 24).

DISCUSSION.—The presence of chloroplasts and starch in the sporophytic cells of five genera of the Marchantiales probably is sufficient evidence to warrant the assumption that at least in the majority of forms in this alliance the sporophyte is strongly photosynthetic and hence not completely "parasitic" or "dependent" on the gametophytic phase. While I have been able to observe the presence of chlorophyll in the sporophyte of only one genus of Metzgeriaceae and in only three of the vast assemblage of Jungermanniaceae, there is every indication that the results of more extensive study will not deviate from the present observations. In this connection we cannot overlook Leitgeb's (1879) and Campbell's (1928) reports of chlorophyll in the sporophytes of *Sphaerocarpos*. We may therefore assume for the Jungermanniales and Sphaerocarpaceae also some degree of nutritional independence of the sporophyte from the gametophyte. A survey of the literature indicates that the problem of nutrition of the hepatic sporo-

phyte has received very little attention per se and, as noted at the outset, only *Anthoceros* and its relatives are usually recognized as possessing some degree of photosynthetic enterprise. Why such an obvious attribute as the color of developing sporophytes in the Marchantiales, Jungermanniales, and Sphaerocarpaceae should have escaped notice is difficult to understand; it is more probable that, though it has often been observed, its significance was not appreciated. In many cases, however, the question of color has been neglected, since the material used by various investigators was immediately preserved and the development studied from sectioned and stained material. With this in mind I have searched through the publications of some of those investigators whose work antedates the development of modern technical methods, such as those of Mirbel (1835) and Hofmeister (1862). Mirbel recognized the presence of chlorophyll in the developing sporophyte of *Marchantia*, as is shown by his figures and the text, and he states, speaking of the elaters and the spore mother cells: "Les unes et les autres étaient encore remplies de sphéroles vertes." Although he described the ontogeny of the sporophytes of many Marchantiales and Jungermanniales, it is only in the case of *Jungermannia divaricata* that Hofmeister (1862) directly mentions the presence of chloroplasts in the sporophyte capsule, and he figures these clearly. His figures of other genera, however, suggest that he may have observed chloroplasts in other sporophyte cells such as in the spore mother cells of *Pellia*, the immature elaters of *Fossombronina* and in *Radula*, *Frullania*, and *Targionia*. Leitgeb (1874-1881) mentions the presence of chlorophyll only in the sporophyte of *Sphaerocarpos*.

Since the sporophytes of the Marchantiales, Jungermanniales, and *Sphaerocarpos*, along with those of the Anthocerotales are regularly photosynthetic and elaborate at least some of their carbon compounds during their metabolism, we are faced with the task of reviewing our conclusions as to the phylogeny of the Hepaticae, and this task will inevitably lead us to the broader question of the relation of these new facts to the question of alternation of generations.

In his discussion of the relationship of the Bryophyta, Bower (1935) stresses as a fundamental criterion the division of labor in sporophyte and gametophyte of vegetative (nutritional) and reproductive processes, pointing out that it is a primitive character for these to be carried on simultaneously and in the same part of the plant; segregation of these functions, culminating as it seems to in the flowering plants, is an advanced or specialized condition. Applying this criterion to the Hepaticae, Bower singles out the Anthocerotales as illustrative of lack of segregation in both gametophyte and sporophyte; as noted above, he stresses the absence of photosynthesis from the sporophytes of the remaining liverworts. In the light of my own observations, it is clear that also in the sporophytes of the Marchantiales and Jungermanniales and in *Sphaerocarpos*, as well as in *Anthoceros*,

we find an absence of segregation of vegetative and reproductive functions—indeed, even in these forms all cells of the sporophyte seem to carry on both functions, except in those cases where the sporocytes themselves are colorless. If foot, seta, and elaters do represent structures which have originated by sterilization of potentially sporogenous tissue, the presence of chlorophyll in them possibly indicates the occurrence of photosynthesis in the primitive archesporium. The situation in these sporophytes is not without analogy to the condition observed in (the gametophytes of) the colonial Volvocales where all the cells are vegetative, but a few (the eggs and daughter-colony-initials) are reproductive as well, while others (the sperms) are probably entirely reproductive, aside from carrying on the function of respiration. Employing segregation of these functions as the only criterion, it is impossible to distinguish among the sporophytes of the four orders of hepatics. Some will argue that the *Anthoceros* sporophyte is more actively photosynthetic than the others, as evidenced by the presence of functional stomata, while these are lacking in the capsules of the other three orders. This can well be interpreted in a manner similar to that in which we explain the absence of stomata in the gametophytes of the Jungermanniales and Sphaerocarpaceles and their presence in the Marchantiaceae—as adaptations to environmental demands. The walls of the epidermal cells in the *Anthoceros* sporophyte seem to be relatively impervious to liquids; Smith (1938) states that they are heavily cutinized, and this is probably correlated with their exposed position; stomata here are a necessity. On the contrary, the sporophytes of the Jungermanniales, Marchantiales, and Sphaerocarpaceles are buried throughout their ontogeny within the protective layers of the venter (calyptra) and, in some cases, of the pseudoperianth and other involucreal tissues. These sporophytic cells are apparently not cutinized, and so they are able to absorb gases directly when these are dissolved in the water on the superficial cell walls. The sporophytes of the Marchantiales, Jungermanniales, and Sphaerocarpaceles are in this respect homoplastic with the gametophytes of the Jungermanniales, Anthocerotales, and Sphaerocarpaceles; those of *Anthoceros* parallel the adaptations observable in the Marchantiacean gametophyte.

Although Goebel (1930, p. 913) regards the Hepaticae as a polyphyletic group, he apparently considers the sporophyte of *Anthoceros* as a primitive, generalized type. Assuming that the intercalary meristem of this type of sporophyte underwent a change in degree and type of activity, he would derive the Marchantiacean and Jungermannian type from it largely on the basis of reduction. In support of such a view he lists such evidences as degeneration of the stomata and assimilatory tissue, decrease in size, suppression of the columella with consequent fertility of the endothecium, suppression of the stalk, elaters, and of a regular dehiscence-mechanism. Whether or not this viewpoint is tenable in its entirety, the

present report of widespread occurrence of chlorophyll in the sporophytes of the Marchantiales and Jungermanniales has an important bearing on Goebel's hypothesis.

The phenomena of widespread occurrence of chlorophyll in the hepatic sporophyte is of interest when it is examined in the light of the theories of the origin of the alternating generations. The fact that many hepatic sporophytes have been considered to be colorless, non-photosynthetic, "parasitic," and "dependent" naturally affords support to the interpolation theory of alternation, according to which the sporophytic and gametophytic phases are fundamentally distinct, the former being interpreted as innovations in the hitherto purely gametophyte cycle. Thus Campbell (1928) and Bower (1935) are inclined to interpret photosynthetic sporophytes in the light of the hypothesis which ascribes the origin of the vegetative tissues in the sporophyte to a secondary sterilization of potentially sporogenous tissue. On the other hand, the present observations which indicate the general occurrence of photosynthesis in the sporophytes of the Hepaticae may be interpreted as evidence in support of the homologous or transformation theory of alternation recently stressed anew by Eames (1936), for the occurrence of photosynthesis in simple sporophytes serves to emphasize the fundamental similarity in capacity for self nutrition of the two alternating phases. Bower (1935), in discussing the ontogeny of the sporophyte in the Bryales, states that in these forms the sporophyte becomes photosynthetic only relatively late and at the time that sporogenesis is in progress. While the present paper includes no report of special study of the problem of nutrition in moss sporophytes, the writer has recently had the opportunity to examine several species in this connection. In *Pogonatum pennsylvanicum* (Hedw.) Paris the young spindle-shaped sporophyte when about 2 mm. in length and still enclosed within the calyptra and gametophore leaves is deep green in color and apparently actively photosynthetic. Certainly in the vast majority of the Bryales the sporophyte during the needle-like, elongating stages is chlorophylliferous and photosynthetic, and this stage considerably antedates that of sporogenesis.

We may well raise the question whether or not there occur among our extant plants or ever did occur in extinct forms any sporophytes which lacked entirely the capacity for autotrophic nutrition by photosynthesis. Although there are those who, like Bower (1935), hesitate to homologize the life cycles of the algae and Archegoniates, it seems clear that the zygotes of those Chlorophyceae with zygotic reduction correspond in ultimate origin, position in the life cycle, and in function. Such a view in no sense conflicts with Oltmann's (1923) insistence that the phenomenon of alternation in algae and Archegoniates is an example of homoplasmy. We may even go so far as to compare these zygotes to a simple sporophyte composed of but a single sporocyte, which sporophyte in the isogamous algae is sufficiently autotrophic by

photosynthesis to provide nutriment for the development of four (*Ulothrix*) or more (*Coleochaete*) zoospores. It is most appropriate to stress the isogamous algae in this connection, since there is often abundant nutriment stored in the female gamete of heterogamous forms. The conclusion is not forced, it seems to me, that the sporophytic phase, whatever its origin or extent in the life cycle, has always been photosynthetic. This conclusion is not contrary to the cytological evidence which indicates that plastid primordia are regularly present at least in the female gamete. If the sporophyte of *Ricciocarpus natans* should be proven by further study to be devoid of chlorophyll, this circumstance can best be interpreted as a physiological evidence of reduction and should perhaps be added to the morphological evidences listed by Goebel (1930). The entire archegoniate series requires investigation with special reference to the nutritional relations between the alternating generations. The capacity for self nutrition by photosynthesis was apparently not sacrificed by the gametophytes of all heterosporous plants when the megaspore became permanently retained in the megasporangium; while the *Pinus* female gametophyte is quite colorless, that of *Ginkgo biloba* is strongly chlorophylliferous throughout the major portion of its ontogeny, in spite of its position deep within the nucellus and the thickened integumentary layers. The physiological relations between sporophyte and gametophyte seem to be to some extent independent of morphological factors.

SUMMARY

The living sporophytes of *Marchantia polymorpha*, *Asterella tenella*, *Dumortiera hirsuta*, *Mannia rupestris*, *Pellia epiphylla*, *Frullania* sp., *Cephalozia* sp., and *Blepharostoma trichophyllum* are strongly photosynthetic during their ontogeny as evidenced by the occurrence of chloroplasts in the immature foot cells, seta cells, capsule wall cells, and elaters.

It is therefore no longer possible to segregate the orders of Hepaticae on the criterion of occurrence of photosynthesis in the sporophyte, since this attribute has been described for certain genera in all the orders.

It is suggested that the capacity for self nutrition by photosynthesis is a primitive character of the sporophytic phase and not a secondary attribute assumed during the sterilization of sporogenous tissue.

Physiological relations between sporophyte and gametophyte do not always parallel morphological characters, as illustrated, for example, by the occurrence of a strongly photosynthetic female gametophyte in *Ginkgo biloba* and a completely "dependent" and colorless one in *Pinus* and probably also in *Zamia*.

The widespread occurrence of photosynthesis in simple sporophytes may be interpreted as evidence in favor of the theory which stresses the fundamental similarity of the alternating generations (homologous theory) rather than their antithetic nature.

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QUANTITATIVE ISOLATION OF CHLOROPLASTS FROM HIGHER PLANTS¹

Sam Granick

CHLOROPLASTS, BECAUSE of their fundamental role in photosynthesis and their prominence in leaf cells, have been the object of many cytological and microchemical studies. A considerable and controversial literature has accumulated on the constitution of chloroplasts, containing a large number of conjectures and a paucity of facts—a condition arising from the limitations of the methods that have been used.

The writer has found that chloroplasts can be isolated by differential centrifuging in amounts sufficient for quantitative determinations. Once the isolation is made, analytical procedures can be taken almost bodily from the field of blood chemistry. It is hoped that the quantitative isolation of chloroplasts will open up a new and more fruitful field of investigations, not only on the composition, structure, and metabolism of these bodies, but also on the mechanism of photosynthesis.

FACTORS INVOLVED IN THE ISOLATION OF CHLOROPLASTS.—During preliminary studies of leaf protoplasm it was noted that partial separations could be made of its components by centrifugation. Examination of the centrifugates of a tissue mash prepared by grinding leaves in 0.5 M glucose solution indicated that the cell wall debris and the unbroken cells were thrown down first, followed in order by the larger crystals, and starch grains torn from the chloroplasts, coagulated cytoplasm, smaller crystals and starch grains, chloroplasts, and finally by very tiny granules. This led to an attempt to isolate chloroplasts in as normal a state and with as little contamination as possible.

It is intended in a later paper to discuss in detail the composition and morphological structure of the chloroplasts. It will suffice here to mention briefly a few facts relevant to the problem at hand. Chloroplasts of tomato and tobacco leaves possess a semi-permeable membrane and can be maintained for a time in a solution which is isotonic or hypertonic with that of the cytoplasm of the leaf cell. Of the various substances tested, 0.5 M glucose or sucrose solutions were found to be the most satisfactory in which to disperse the chloroplasts. These solutions do not coagulate the cytoplasmic proteins nor cause coarse granules to appear in the chloroplasts, as do salt solutions in general. The chloroplasts obtained in this manner remain normal for several hours as judged from observations on the presence of functional semi-permeable membranes, although very fine

granules become visible in them after a short time. They have been found to produce oxygen for several minutes in the presence of light, as determined on individual chloroplasts by Engelmann's motile-bacteria method. The nuclei, at the pH of the tissue suspensions (pH 6.0-6.5), disintegrate completely and so do not come down with the chloroplasts on centrifuging.

In order to decide whether careful microscopic observation of the centrifugate might be used as a criterion of the purity of the chloroplasts which had been separated out, analyses were first made on the nitrogen content of these bodies. It will become clear further on that because of the method of analysis used, if cytoplasmic proteins were carried down with the chloroplasts, a value for the percentage of N of the chloroplasts in a cell as compared with that of the total N per cell might well exceed 100 per cent. From the nitrogen analyses it was found that the lowest and most consistent N values were obtained for the cleanest chloroplast centrifugates as revealed by microscopic examination under an oil-immersion lens. It appears therefore that direct microscopic examination is a reliable method for checking the purity of the isolated chloroplast fraction. Furthermore, certain experiments now in progress on the enzyme content of chloroplasts lead the writer to the belief that there is relatively little contamination of the isolated chloroplasts with other proteinaceous materials of the protoplasm.

No definite statement can be made concerning the rate of centrifuging, since this was found to depend on the age and kind of leaf that was used. However, it may be said that as a general rule centrifugation at 400 to 800 times gravity was found satisfactory.

The method is not applicable to the extremely young leaves in which cell division is still taking place, because it is difficult to isolate the small chloroplasts from the protoplasm. Also, determinations cannot be made on the oldest leaves which are yellow and have degenerating chloroplasts, since not only does the protoplasm appear to coagulate very readily, but the chloroplasts have too little pigment to permit of their quantitative estimation by use of the colorimeter. These factors likewise make the analyses of the earliest and latest stages of growth somewhat less accurate than those made on leaves that have just attained their maximum size. A further limitation of the method is the fact that it is necessary to choose leaves whose cell walls are readily broken by maceration in sand so that the chloroplasts can float out into the sugar solution. Of a number of leaves tested, those of tomato and tobacco were found especially suitable.

FACTORS INVOLVED IN THE QUANTITATIVE ESTIMATION OF THE CHLOROPLASTS.—In order to determine the percentage of the chloroplasts isolated, one may

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count the number of chloroplasts in the centrifugate and compare this count with the number of chloroplasts in the gram of leaf. Such a method is obviously impractical. An indirect method is used for this determination which takes advantage of the fact that the chloroplast pigments are present solely in the chloroplasts. It is assumed that the quantity of chloroplasts is directly proportional to the concentration of chloroplast pigments contained in them. This assumption is valid for all practical purposes since we are dealing with a large number of chloroplasts. In order to illustrate this principle, let us suppose that a fraction, obtained from one gram of leaf, is centrifuged down and on microscopic examination is seen to contain only chloroplasts. An acetone extract is then made of these chloroplasts. At the same time, one gram of leaf material is also extracted with acetone. On comparing the extracts, it is found, let us say, that the extract of chlorophyll pigments of the isolated chloroplasts contains one-tenth of the chlorophyll pigments in one gram of leaf. Then it is justifiable to assume that ten per cent of the chloroplasts in one gram of leaf are present in the centrifuged fraction.

The same principle is utilized in another way. Suppose a suspension of chloroplasts is centrifuged for a short time. Then a certain amount of the chloroplasts will be thrown to the bottom of the tube. If the concentration of chloroplast pigments in the suspension is determined before and after centrifuging, then the concentration of chloroplast pigments in the centrifugate may be calculated.

In order to determine the concentration of plastid pigments, extractions of the suspensions are made with 75 per cent acetone, which has been found to be better than a number of other solvents for this purpose. The acetone dissolves the pigments of the chloroplasts and at the same time produces a heavy flocculent precipitate of the proteins. On centrifuging, a clear green supernatant solution results which can be used for colorimetric determinations. A clear solution of the pigments is essential, since the accuracy of the chloroplast values depends on the accurate determination of the concentration of the pigments in the aliquots of the chloroplast suspensions before and after centrifuging.

Although preliminary experiments showed that the acetone extracts of the pigments of most leaves fade slowly, the fading was found to be directly proportional to the concentration of the pigments. Since the method is not concerned with the absolute but with the relative concentrations of the pigments, this introduces no error. However, experience with a large number of analyses has indicated that occasionally the fading of acetone extracts of the pigments is not proportional to the concentration. In order to avoid errors due to fading, it has been found best to make extractions with ice cold acetone and to determine the pigment concentrations immediately after the preparation of the acetone extracts. Extracts of the pigments can also be made with a 3:1

alcohol-ether mixture. These extracts fade less rapidly than do the acetone extracts but sometimes do not give as clear solutions.

SAMPLE ANALYSIS.—An example from a typical analysis will best illustrate the method of isolating the chloroplasts and the method of calculating the fraction of the total chloroplasts that has been isolated. Of the various modifications of procedure tested, experience suggests the following ones as the most satisfactory:

An average sample of three grams of fresh leaf tissue, excluding midribs and main veins, is weighed out rapidly on a torsion balance. The tissue is then kept between moist toweling paper until ready for analysis. During the interim, the cells absorb water, becoming turgid, and in this condition are more readily torn apart. Analyses are generally begun within an hour after the leaves have been cut from the plants.

The leaf tissue is removed from the moist toweling paper, washed once with distilled water, superficially dried, and a portion of it placed in a deep porcelain mortar of about 150 cc. capacity. One gram of quartz sand and 25 cc. of 0.5 M glucose solution at a temperature of about 5°C. are added to the mortar. The tissue is then rubbed gently with the pestle against the sand in order to tear the turgid cells apart and release the chloroplasts into the cold hypertonic glucose solution. When the solution has become dark green, the flaccid tissue is squeezed against the side of the mortar, and another portion of tissue is added and the cells torn apart in a similar manner. The chloroplast suspension is decanted into a 50 cc. round-bottom centrifuge tube. The remainder of the tissue is then ground in an additional 20 cc. of the cold glucose solution, and this chloroplast suspension is added to the first in the centrifuge tube. Thus, a total of approximately 45 cc. of a green suspension is obtained. (The leaf residues remain in the mortar and are treated as mentioned below.)

As mentioned above, the time and rate of centrifugation that are used in the isolation of the chloroplasts depend on the leaf material under investigation. These factors must be controlled by careful examination of the centrifugates under an oil-immersion lens. The time intervals for centrifuging that follow are taken from a typical analysis.

The suspension is centrifuged for some seven minutes at about 400 times gravity in order to throw down any heavy cellular debris, crystals, and large starch granules. The supernatant suspension is then decanted into another centrifuge tube and diluted up to a 45 cc. mark with glucose solution. This suspension is designated as suspension A. Five cc. of suspension A are pipetted into a tapering-bottom centrifuge tube of 20 to 25 cc. capacity for the determination of the concentration of chloroplast pigments.

The debris remaining in the first centrifuge tube is transferred to the mortar and, together with the leaf residues, is ground up very thoroughly in glucose solution and made up to 50 cc. in a volumetric flask.

This tissue mash is designated as suspension D. Five cc. of this suspension are pipetted into a centrifuge tube for the determination of its concentration of chloroplast pigments.

The remaining 40 cc. of suspension A are centrifuged for five to eight minutes. The resulting supernatant suspension is then decanted into another centrifuge tube and is designated as suspension B. The chloroplasts which have been thrown down are designated as centrifugate B and serve for the direct analysis of nitrogen, phosphorus, or enzyme, etc. Five cc. of suspension B are pipetted into a centrifuge tube for the determination of the concentration of chloroplast pigments in suspension B.

The 35 cc. of suspension B remaining are further centrifuged for five to ten minutes, the supernatant suspension decanted into a test tube and designated as suspension C. The chloroplasts that have been centrifuged down are designated as centrifugate C and serve for direct analysis. Five cc. of suspension C are pipetted into a centrifuge tube for the determination of the concentration of chloroplast pigments.

To each of the 5 cc. samples of suspensions A, B, C, and D, which have been transferred to the tapering-bottom centrifuge tubes of 20 to 25 cc. capacity, are added 10 to 15 cc. of ice cold acetone. By properly tapping the tubes to set up a swirling motion, the solutions will be found to mix thoroughly. They are placed in a beaker containing some ice and water in a dark place for some ten to fifteen minutes. Then the tubes are stirred again and centrifuged rapidly for five minutes. Crystal-clear green solutions result. These are decanted into 25 cc. volumetric flasks, marked respectively A, B, C, and D, which are placed in ice water. The residues in the centrifuge tubes are extracted once more with 5 cc. of 75 per cent acetone and the extracts added to the main volume of extracts in the flasks. The green solutions are compared in a Duboscq biological colorimeter. Since much depends upon the accuracy of the readings of the colorimeter, the instrument should be carefully adjusted and duplicate readings should not vary by more than 0.2 mm. on the scale. Beer's law is obeyed within the range of concentrations that are used.

Comparisons are made between solutions A and B, B and C, and A and D. The scale readings of the cups of the colorimeter are given for a typical analysis: $A/B = 21.1/30.0 = 0.702$; $B/C = 20.4/30.0 = 0.679$; $A/D = 20.0/21.1 = 0.942$.

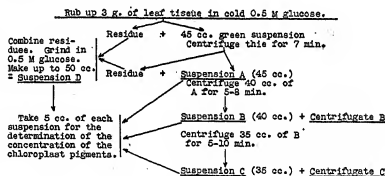
CALCULATION OF THE PERCENTAGE OF THE CHLOROPLASTS PRESENT IN CENTRIFUGATES B AND C.—In order to calculate the percentage of the chloroplasts present in centrifugates B and C, it is necessary to know the percentage of the pigments present in suspension A. This may be determined in the following manner:

All the blade material taken for analysis (3.0 g.) is present in 45 cc. of suspension A + 50 cc. of suspension D. Consider that the amount of pigments in the sample is 100 per cent. Let the concentration of pigments in 1 cc. of suspension A = 1.0 unit.

Now $A/D = 0.942$ —i.e., 1 cc. of suspension D is only 0.942 times as concentrated as 1 cc. of suspension A. Then $A = 1.0 \times 45 \text{ cc.} = 45.0$; $D = 0.942 \times 50 \text{ cc.} = 47.2$ and the sum i.e., $45.0 + 47.2 = 92.2$ units \approx 100 per cent of the pigments. The percentage of the pigments or of the chloroplasts which are present in 45 cc. of suspension A = $45 \times 100/92.2 = 48.8$ per cent. Before suspension A is centrifuged, 5 cc. are removed, leaving only 40 cc. of A. The percentage of chloroplasts present in 40 cc. of A is $40/45 \times 48.8 = 43.4$ per cent. After centrifuging, the percentage of chloroplasts present in 40 cc. of suspension B = $A/B \times 43.4$ or $0.702 \times 43.4 = 30.5$ per cent. The percentage of chloroplasts in centrifugate B is equal to the difference in the percentage of pigments before and after centrifuging the 40 cc. of chloroplast suspension—i.e., 43.4 per cent — 30.5 per cent = 12.9 per cent. Centrifugate B therefore contains 12.9 per cent of the total number of chloroplasts present in three grams of leaf.

The percentage of chloroplasts in centrifugate C is calculated in a similar manner. In 40 cc. of suspension B, 30.5 per cent of the chloroplasts are present. In 35 cc. of suspension B, 26.7 per cent of the chloroplasts are present. After centrifuging, suspension C contains 0.679×26.7 per cent = 18.1 per cent of the chloroplasts. The percentage of chloroplasts in centrifugate C is then $26.7 - 18.1 = 8.6$ per cent. Centrifugate C therefore contains 8.6 per cent of the total number of chloroplasts present in three grams of leaf.

An outline of the method for the quantitative isolation of chloroplasts is given in the accompanying diagram.



An example, taken from some Kjeldahl nitrogen determinations will illustrate the method of calculating the percentage of nitrogen in the chloroplasts (table 1). The table is self-explanatory.

If nitrogenous substances, which are not a part of the chloroplasts, are carried down with them, it will generally be found that centrifugate B will give a higher value for the percentage of nitrogen in the chloroplasts as compared to centrifugate C. The two centrifugates serve as a check against each other. It will be observed from the table that the values obtained from both centrifugates, as indicated in the last column, check each other within the limits of the experimental error of the procedure.

A second procedure, which is also quite satisfactory, is the following: About ten grams of leaf blade

material are ground up to release the chloroplasts, as described above. A first short centrifuging removes the debris. A second more rapid and prolonged centrifuging carries down most of the chloroplasts.

These chloroplasts are then resuspended in glucose solution and centrifuged for a short time to remove clumps of chloroplasts. A second, more prolonged centrifuging, carries down most of the chloroplasts in a relatively pure condition.

the cytoplasmic proteins coagulate. The chloroplasts are separated from other cell materials by a system of differential centrifuging.

A method is presented for relating the quantity of chloroplasts isolated to the total quantity of chloroplasts in the leaf sample. The method is based on the assumption that the number of chloroplasts is directly proportional to the quantity of chloroplast pigments contained in them. By determining the

TABLE 1. *Method of calculating percentage of nitrogen in chloroplasts.*

Material	% of the chloroplasts used for analyses	N in the chloroplasts	N in all (100%) of the chloroplasts	% of the total-N in the leaf blade material which is present in the chloroplasts
Centrifugate				
B	12.9%	0.917 mg.	7.12 mg.	43.8%
C	8.6	0.578	6.72	41.5

The cleaned chloroplasts are then suspended in distilled water and analyses are made on them. An aliquot of the suspension is taken for the determination of the concentration of chloroplasts in the suspension. The aliquot is treated with acetone in a centrifuge tube, and the contents of the extracted chloroplast pigments are compared in a colorimeter with the pigments extracted from one gram of fresh leaf material, as described above. The number of chloroplasts in the washed suspension can thus be related to the number of chloroplasts present in one gram of fresh leaf-blade material.

SUMMARY

A procedure is described for isolating chloroplasts from tomato and tobacco leaves. The cells of the leaves are torn apart by grinding the cells under hypertonic sugar solutions. The suspended chloroplasts in these solutions do not disintegrate, nor do

amount of chloroplast pigment in a fraction of isolated chloroplasts and relating this value to the amount of chloroplast pigment in the entire tissue sample, one may obtain the percentage of pigment present in the fraction of isolated chloroplasts. For practical purposes this will also be the percentage of chloroplasts which is isolated from the tissue sample.

A certain quantity of chloroplasts are isolated in a relatively pure state from three grams of leaf material, and the pigments of these chloroplasts are extracted with 75 per cent acetone. The quantity of pigments extracted from them is then compared colorimetrically by means of a Duboscq colorimeter with the quantity of pigments extracted from three grams of leaf material. From this value one may obtain the percentage of the chloroplasts which have been isolated from three grams of leaf material.

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CHLOROPLAST NITROGEN OF SOME HIGHER PLANTS¹

Sam Granick

IN THE first paper of this series (Granick, 1938) a method was described by which chloroplasts can be isolated quantitatively. In the present paper, the method is applied to the analyses of the nitrogenous composition of these bodies. Data on the nitrogenous constituents, especially the proteins, have long been desired in order to answer several important questions concerning the physiology of these bodies—namely: To what extent are the proteins of a cell

present in the chloroplasts? Are the chloroplasts significant synthesizers of proteins? What is the relation between the protein of the chloroplasts and chlorophyll?

The investigations as far back as Sachs (1862) and the more recent ones of Molisch (1916), Lakon (1916), Meyer (1918), Ullrich (1924), and others indicated that the major portion of the cell proteins of the leaves was present in the chloroplasts. The changes observed in the size of these bodies on supplying starved leaves with a carbohydrate and nitrogen source, or accompanying the aging of leaves, led these workers to the further conclusion that the major portion of the cell proteins of the leaves is synthesized by the chloroplasts. Schumacher (1929) has recently criticized the above mentioned investigations

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on the ground that the interpretations were based on rough protein staining reactions and inaccuracies in the measurements of the size of the chloroplasts. He reports that the size of the chloroplasts in *Pelargonium* is not directly proportional to the protein content of the leaf, although large fluctuations in protein nitrogen of the leaf do appear to affect the size of the chloroplasts. He believes that only a relatively small proportion of protein nitrogen can be present in the plastids. He likewise minimizes the activity of the plastids in protein synthesis.

These diametrically opposed conclusions concerning the protein content of chloroplasts are clearly the result of the inadequacy of the methods that have been used. By isolating chloroplasts in sufficient quantities for direct microchemical analyses an unequivocal answer can be given to these problems.

The work of Menke (1938) should be mentioned in this connection. By grinding up spinach leaves in water over a period of several hours and adding to this suspension a certain concentration of ammonium sulphate and then acidifying slightly, Menke obtained a green insoluble fraction containing most of the lipoids, which he designates as "chloroplast substance," and a soluble protein fraction, the "cytoplasmic substance." These fractions are strikingly similar to those reported by Chibnall and Grover (1926) in a study of leaf cell cytoplasm. They found that by grinding leaves in water, a fine suspension of cytoplasmic constituents could be obtained which could be separated on filtration through a thick layer of paper pulp into an insoluble "combined protein" fraction which is in loose combination with substances soluble in alcohol and a colorless "soluble protein" fraction. The "chloroplast substance" of Menke is evidently closely related if not identical with the "combined protein" of Chibnall and Grover.

There is little doubt that a large portion, if not all, of the protein of the chloroplast is present in the "chloroplast substance" of Menke, but there is considerable doubt as to whether that fraction represents solely the protein of the chloroplasts. It has been my experience with aqueous suspensions of tobacco and of tomato leaf, which have been centrifuged to remove the coarser cellular debris, that such suspensions are extremely labile and at times very sensitive to the addition of even minute quantities of neutral salts. Under the microscope the precipitate which results can be seen to contain many smaller and partially disintegrated chloroplasts embedded in a mashwork of protein, the precipitate appearing to be evidently very much more than chloroplast protein.

The data of Chibnall and Grover indicate that there may be a greater or lesser proportion of "combined protein" to "soluble protein" of the leaf, depending on the species of leaf examined. Menke's analyses show that the protein of the "chloroplast substance" is 11.1 per cent and the protein of the "cytoplasmic substance" 12.1 per cent of the dry weight of the leaves—i.e., a nearly 1:1 ratio. Chibnall and Grover analyzed four species of leaves for "combined" and

"soluble protein." In three of the species (their table 5)—namely, *Cochlearia amoralis*, *Brassica oleracea*, and *Spinacia oleracea*—the ratio of "combined" to "soluble protein" is nearly 1:1. However, in *Phaseolus multiflorus*, there appears to be more than twice as much "combined" as "soluble protein." If Menke had used *Phaseolus*, his yield of protein of the "chloroplast substance" might have been twice as great as with *Spinacia*.

It appears therefore that in order for Menke to relate the protein of the "chloroplast substance" to the protein of the chloroplasts themselves, it will be necessary for him to show that there is little or no cytoplasmic protein present in his "chloroplast substance." It will likewise be necessary to show that the ratio of the "chloroplast substance" to the "cytoplasmic substance" is the same in the macerate as it is in the cells.

METHODS AND ANALYSES.—Methods of sampling.—After plants such as tomato and tobacco have become well established, and before the fruit is set, the leaves then developing attain approximately the same size when fully matured, and presumably possess the same number of cells. Under such conditions one may compare parenchyma cells of different physiologic ages by analyzing leaves at successive nodes below the tip of the plant. Since rather small amounts of leaf tissue are taken for analysis, and since it is advisable to take as many leaves as possible in order to secure a good average sample, a description of the method of sampling tomato leaves is given here. The same method with a few minor variations has been applied to the sampling of tobacco leaves.

For each analysis some ten or more similar leaves of different plants are carefully chosen with respect to their position on the stem, their length, and the number and size of their leaflets. All weighings are made rapidly on a torsion balance. The average fresh weight per whole leaf is first determined. The midribs of the leaves and the main veins of the leaflets are then cut out quickly with a sharp razor blade and are discarded; the remainder of the leaf is designated as leaf blade material. The leaflets of one half of a leaf (e.g., the right half) are stacked in a separate pile from those of the other half. On weighing out a sample, portions from each stack of leaflets, coming, for example, from the right half of each leaf, are cut lengthwise and placed on the balance until the required weight is secured. Inasmuch as the total length of time in weighing (less than 5 minutes) is less than the time taken to prepare the tissue (some 10 to 15 minutes), the weight of the total leaf blade material is determined after weighing the samples, and from this value is calculated the weight of leaf blade material per leaf. If sampled and calculated in this manner, the error in weighing which is the result of drying is not more than one per cent.

Method of nitrogen determination.—Since small amounts of nitrogen were to be determined, the

ammonia produced in Kjeldahl digestion was Nesslerized and determined colorimetrically with a Duboscq colorimeter. Nitrate-N was not determined. An attempt was made to determine the ammonia directly in the sulfuric acid digest by using $K_2S_2O_8$ without having recourse to ammonia distillation. However, it was found that not only were digestion and clearing extremely slow, but also turbid Nessler solutions resulted. Digestion with a sulfuric acid-phosphoric acid mixture was not much better. The procedure finally adopted was to digest the leaf material in 100 cc. Kjeldahl flasks containing sulfuric acid and potassium sulfate in the proportion of 2 cc. of the acid to 1.0 gram of the sulfate with the addition of a few crystals of copper sulfate. Clearing took place in 5 to 15 minutes. Digestion was further continued for about one-half of the time it took to clear in order to decompose any resistant pyrrole rings. The ammonia was then distilled with the aid of steam (Peters and Van Slyke, 1932) into dilute acid and Nesslerized. Checks on the method with known amounts of ammonia gave recoveries of 98 to 101 per cent.

Experiments on the tobacco leaf.—The tobacco plants of the variety Maryland Mammoth were grown in a greenhouse. They were used in the autumn when they were three to four feet tall and in a vigorous vegetative condition, bearing succulent dark green leaves. Leaves of similar appearance and from similar nodes were taken from the plants between 8 and 9 a.m. These were sampled as described above. The method of isolating the chloroplasts from the leaves and the method used in calculating the data are described in the first paper of this series.

It is seen from figure 1 and table 1 that there is an increase of total-N and chloroplast-N as the young leaf increases in size until it attains its fully expanded, mature state. Then follows a progressive decrease in these nitrogen values as the leaf becomes older. The increase in total nitrogen of the leaf material per leaf, as the leaf expands from the first stage (2.26 mgm. N) to the second stage (25.6 mgm. N), is very striking. During this time the rate of increase of nitrogen in the leaf and in the chloroplasts is so great that it keeps pace with the rate of increase in fresh weight of the leaf.

Especially significant is the fact that the chloroplasts contain between 30 to 35 per cent of the total nitrogen of the entire leaf blade material at all the stages of growth examined. In order to determine how much this increase in nitrogen was the result of an increase in protein, finely ground leaf material was treated with 7 per cent trichloroacetic acid at 80°C. for ten minutes. The nitrogen, insoluble in this reagent, is principally protein nitrogen. In table 1, data are presented of the nitrogen fraction of the leaf that is soluble in trichloroacetic acid. From these figures it appears that approximately 80 per cent of the total Kjeldahl nitrogen of these leaves may be considered as protein nitrogen. In the younger leaves there is somewhat less soluble-nitrogen present in the trichloroacetic extract (17.8 per cent) than in the

older green leaves (22 per cent). In old, degenerating leaves the level of the soluble nitrogenous constituents is high (28.3 per cent), probably indicating a lessened ability of the protoplasm to synthesize proteins.

Experiments on the tomato leaf.—John Baer tomato plants grown in the greenhouse were used for analyses during the spring months, at which time they were from four to six feet tall. A sufficient number of plants was available so that plants of similar appearance could be readily selected. All the leaves examined had seven prominent leaflets. In order to designate in table 2 the node from which the leaf arises, the first node is arbitrarily considered as that node at the top of the plant from which a leaf more than five centimeters in length arises. In turn, the larger leaves immediately below this are considered as arising from the second node, third node, and so forth.

The data of table 2 show that the percentage of total nitrogen, present in the chloroplasts at all stages of growth, ranged between 30 and 40 per cent of the total nitrogen of the leaf blade material. The figures

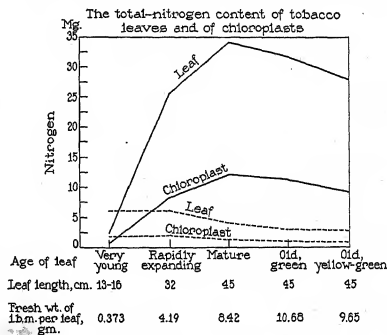


Fig. 1. The solid lines of the graph represent the nitrogen in terms of a single leaf, exclusive of its vascular tissue. The broken lines represent the nitrogen in terms of a gram fresh weight of leaf, exclusive of vascular tissue.

in the last column of the table represent duplicate determinations on two successive chloroplast centrifugates and indicate the relative accuracy that may be expected from these analyses. A comparison of the data on the basis of the cells of the tomato leaves of these plants is not strictly justifiable, since the old leaves which were used were on the plants when they were transplanted to larger pots. From the first column of table 2 one may observe that the younger leaves which developed subsequent to the repotting were somewhat longer in their fully expanded condition (29-31 cm.) than the old leaves (24-27 cm.). In spite of this fact, the data on the nitrogen of

TABLE 1. *The total-nitrogen content of tobacco leaves and chloroplasts.*

Leaf size and age	Fresh wt. per leaf	Fresh wt. of blade material per leaf	Total-N per g. fresh wt. of leaf blade material	Total-N of blade material of a leaf	Total-N of chloroplasts per g. fresh wt. of leaf blade material	% of total-N present in the chloroplasts	Nitrogen of leaf blade material soluble in 7% trichloroacetic acid—in % of total-N
Young leaves near top of plant 13-16 cm. long	0.625 g.	0.373 g.	6.04 mg.	2.26 mg.	1.81 mg.	30.0%	17.8%
Expanding leaves, 32 cm. long 10 cm. wide	6.76	4.19	6.12	25.6	2.02	33.0	—
Mature leaves 45 cm. long 14 cm. wide	14.21	8.42	4.05	34.1	1.41	35.6	22.0
Old leaves still green (plants in flower) 45 cm. long 16 cm. wide	18.34	10.68	2.96	31.6	1.05	35.5	22.3
Old leaves tinged yellow 45 cm. long 16 cm. wide	16.25	9.65	2.88	27.8	0.912	32.7	28.3

TABLE 2. *The total-nitrogen content of tomato leaves and chloroplasts.*

Leaf size and position on the stem	Fresh wt. per leaf	Fresh wt. of blade material per leaf	Total-N per g. fresh wt. of l.b.m.	Total-N per blade material of a leaf	Total-N of chloroplasts per g. fresh wt. of l.b.m.	% of total-N of l.b.m. present in the chloroplasts
Young leaves at 4-5th node, 26-27 cm. long	3.38 g.	1.29 g.	5.50 mg.	7.62 mg.	2.08 mg. 2.26	37.9 % 41.2
Nearly mature leaves at 6th node, 27-29 cm. long	4.03	1.65	5.40	8.88	2.37 2.13	43.8 39.4
Mature leaves at 7-8th node, 29-31 cm. long	5.42	2.30	3.78	8.72	1.35	35.7
Old leaves at 12th node, 26-29 cm. long, medium to light-green in color	6.70	3.02	2.50	7.68	0.768 0.934	30.7 36.2
Old leaves at 16-18th node (2-3 nodes above the soil), 24-27 cm. long, yellowish-green in color	6.16	2.91	1.62	4.72	0.592 0.562	36.5 34.5

tomato chloroplasts agree very well with those obtained on tobacco chloroplasts.

Several determinations were also made on the nitrogenous fractions of the chloroplasts. Chloroplasts from mature leaves of tomato were extracted several times by successive centrifugations and decantations with a 3:1 alcohol-ether solution at boiling temperature. The chlorophylls and phospholipids were removed by this treatment. The extract was then evaporated and its nitrogen content determined. The residue from the alcohol-ether extract was further treated with 6 per cent trichloroacetic acid, and the soluble nitrogen removed by this reagent was also determined. Finally the nitrogen of the residue re-

maining was determined. This last fraction is considered to represent the protein nitrogen of the chloroplasts. The data of a typical analysis are given in table 3.

The very high protein content of the chloroplasts is evident from table 3 and reveals that the increase of nitrogen in the chloroplasts at various stages of leaf development is due for the most part to an increase of protein in these bodies.

The rather high content (13.2 per cent) of the alcohol-ether fraction is interesting. It appeared desirable to determine whether the nitrogen of this fraction represented other compounds besides those of the green pigments. The chlorophyll content of

this fraction was determined colorimetrically by converting the green pigments into the water-soluble chlorophyllins and comparing them with the chlorophyll standard of Guthrie (1928) in a Duboscq colorimeter. From a number of such determinations on mature tomato leaves, it was found that approximately 10 per cent of the total nitrogen of the chloroplasts is present in the chlorophylls A and B. Since 13.2 per cent of the nitrogen of the chloroplasts is found in the alcohol-ether extracts, it is evident that some other nitrogen-containing material, possibly a phosphatide, is present in this extract and accounts for the additional 3 per cent of the nitrogen in this fraction. In the next paper of this series data will be presented which support this conclusion.

TABLE 3. *The nitrogen fractions of isolated chloroplasts.*

Treatment	% of total Kjeldahl-N
3:1 alcohol-ether extract (Chlorophylls and phospholipids)	13.2%
Residue of above extracted with 6% trichloroacetic acid (Amino acids, peptids etc.)	6.7
Residue of above treatments (Proteins)	80.1

DISCUSSION.—*The percentage of protein-nitrogen in specific regions of a leaf parenchyma cell.*—The data which have been presented for the nitrogen content of tomato and tobacco leaves show that in all stages of leaf development which have been examined, the chloroplast nitrogen ranges between 30 and 40 per cent of the total nitrogen of the leaf blade material. In order to relate these nitrogen values to the values of protein-N in single parenchyma cells, certain corrections must be made, since the leaf is not a uniform structure.

The epidermal cells constituting the upper and lower surfaces of the leaves contain very few and tiny chloroplasts (exclusive of the guard cells). If correction for the nitrogen in the epidermal cells is made, then the calculated percentage of chloroplast nitrogen in the parenchyma cells will be greater than the values given above. Exactly what percentage of the total nitrogen of a leaf is present in the epidermal layers it is as yet impossible to say. Studies of living and fixed sections of tomato leaves show that the epidermal cells are relatively large and layered with rather thin protoplasmic films. Assuming that the volume of the protoplasmic films is proportional to the nitrogen content, it can be roughly estimated that about 10 per cent of the non-chloroplast nitrogen of the leaf-blade material is present in the epidermal cells. The corrected value for the percentage of nitrogen in the chloroplasts of parenchyma cells is therefore about 35–45 per cent of the total-N of these cells.

Eighty per cent of the nitrogen in the chloroplasts is protein-N. Then the protein-N of the chloroplasts is approximately 28 to 36 per cent of the total-N of

the parenchyma cells. Since 75 to 80 per cent of the nitrogen of the leaf blade material is protein-N, it may be calculated that about 35 to 45 per cent of the protein-N of an average leaf parenchyma cell of tomato and tobacco is present in the chloroplasts. The localization of protein is therefore not almost exclusively in the chloroplast as Ullrich and others believed, nor is it almost exclusively in the colorless protoplasm as Schumacher believed.

It is not yet possible to say what portions of the remaining 55 to 65 per cent of the protein of a parenchyma cell are present in the cytoplasmic fluid, the nucleus or the central vacuole. Chibnall (1923) reports the analysis of a fraction of spinach leaves which he considered to be vacuolar sap. He found this sap to contain only traces of protein, which amounted to approximately 1 to 2 per cent of the total leaf nitrogen. If it is assumed that the vacuolar sap of tomato and tobacco leaves contain very little protein nitrogen, it may then be said that 55 to 65 per cent of the protein nitrogen of the leaf parenchyma cell is contained in the cytoplasmic fluid and nucleus.

The regions of protein synthesis in a leaf parenchyma cell.—It had early been observed by Schulze and Schutz (1909) that as the young leaf of *Acer negundo* increased in size, its protein content increased. At about the time the leaf had attained its maximum size, its protein content was at a maximum. As the leaf aged, the content of protein decreased gradually. These changes in *Acer negundo* leaves were in general similar to those which were found in tobacco and tomato leaves (fig. 1) in which approximately 75 to 80 per cent of the total-N is protein-N.

Although marked quantitative changes in nitrogen content take place in the leaves as they develop and age, the striking fact presented by the data in tables 1 and 2 is that the chloroplast nitrogen increases or decreases in proportion to the total-N of the leaf. This is shown by the relative constancy of the relation between the nitrogen of the chloroplasts and the nitrogen of the leaf-blade material in the progressively aging leaves. The nitrogen of the chloroplasts ranges between 30 and 40 per cent of the total-N of the leaf blade material of both tomato and tobacco in the various growth stages examined. The chloroplast-N (fig. 1) increases rapidly with the total-N as the leaf expands, reaching a maximum at the time the leaf has attained its fullest size, finally decreasing rapidly as the leaf becomes yellow (table 2). For example, if we consider (table 1) the total-N in the leaf-blade material of a tobacco leaf (i.e., exclusive of midrib and main veins) from the very young stage (when the leaf is one-third of its maximum length) to the next stage (two-thirds of its maximum length), it will be found that a more than ten-fold increase in nitrogen content has occurred (i.e., from 2.26 to 25.6 mgm. N). Avery (1933) has shown that cell divisions do not occur in the tobacco leaf after it has attained one-fifth to one-sixth of its maximum length. Therefore, in the first two stages examined, the increase in

size of the leaf is due solely to an increase in the size of the cells. Since the predominant cells of the leaf are parenchyma cells, it may be said that a parenchyma cell two-thirds the maximum size contains approximately ten times more total-N than a cell one-third the maximum size. From the second stage to the mature, fully expanded stage there was an increase in total-N of only about 25 per cent of the preceding stage (i.e., from 25.6 mgm. N to 34.1 mgm. N), indicating a great slackening in rate of protein synthesis. At the same time, the rate of increase of chloroplast nitrogen slowed up to about the same extent as the rate of increase of nitrogen for the entire parenchyma cell.

The increase of chloroplast-N might be due presumably to an increase in the number of chloroplasts in a cell, or to an increase in the nitrogen content of the individual chloroplasts, or to both of these factors. In order to determine to what extent these factors were involved, counts were made of the number of chloroplasts in the cells of tomato leaves at different stages of growth. It was found that there is approximately a 30 per cent increase in the number of chloroplasts in an average parenchyma cell of tomato leaves from the very young leaf (one-third maximum size) to the fully expanded leaf. If we assume that this increase in the relative number of chloroplasts as the leaves expand holds also for the tobacco leaf, then, considering the first and third stages of tobacco leaf development (table 1), we may estimate the increase of the division of the chloroplasts in relation to the increase in their nitrogen content. A simple calculation shows that if the nitrogen content of the individual chloroplasts remained the same in the mature as in the young stage, then the 30 per cent increase in the number of chloroplasts could account for no more than about 2 per cent of the increase in nitrogen content of the chloroplasts. It must therefore be concluded that an increase in the nitrogen content of the chloroplasts is due, for the most part, to the increase in nitrogen of the individual chloroplasts. This confirms to some extent the observations of Ullrich (1924) and Schumacher (1929) that large fluctuations in the nitrogen content of a leaf are reflected in changes in the relative sizes of the chloroplasts. There appears to be a rough proportionality between the size of the chloroplast and its nitrogen content.

Since the protein nitrogen of the chloroplasts increases as the chloroplasts enlarge, the question arises as to whether or not these bodies possess the ability to synthesize proteins. The fact that a chloroplast contains the chlorophyll pigments and starch indicates that this body is different from the colorless cytoplasm. It may be assumed that conditions and the complexes of enzymes which bring about the synthesis of the pigments and starch are present therefore only in these bodies. A definite semipermeable membrane surrounding the chloroplasts is apparently effective in confining these products. If these assumptions hold for the pigments and for starch, it may

likewise be reasoned that the only explanation for the twelve-fold increase in the protein content of the individual chloroplasts (0.56 to 6.7 mgm. N from the first to the second stages of the enlarging tobacco leaf) is the synthesis of the protein by the chloroplasts. Likewise, the ten-fold increase in the remainder of the protoplasm (i.e., the fluid cytoplasm and the nucleus) is best explained by assuming that the protoplasm, exclusive of the chloroplasts, also possesses the ability to synthesize its own proteins.

The chlorophyll content per chloroplast.—From determinations on the number of chloroplasts and the chlorophyll content in leaves of tomato which had attained two-thirds their maximum length, it can be calculated that the average chloroplast at this stage contained 1.2×10^{-12} g. of chlorophylls. $a + b$. This value is approximately half of that found by Euler, Bergman, and Hellstrom (1934) for the chloroplasts of *Elodea* (2.5×10^{-12} g. of chlorophyll per chloroplast).

The ratio of protein to chlorophyll in the chloroplasts.—The relation between the number of protein and chlorophyll molecules is important in a consideration of the state of chlorophyll in the leaves. Assuming that the average molecular weight of the proteins is 100,000, it can be calculated that there are approximately 30 chlorophyll molecules per protein molecule present in the chloroplasts. The significance of this ratio will be discussed in a later paper.

SUMMARY

Methods are described for the determination of the nitrogenous constituents of the chloroplasts, as compared to the remainder of the protoplasm, during the development and aging of leaf parenchyma cells of tomato and tobacco. The chloroplasts were isolated from the remainder of the protoplasm as described in the first paper of this series. Nitrogen was determined by Kjeldahl digestion, the ammonia being distilled with the aid of steam, and Nesslerized. The nitrogen fractions of the chloroplasts which were analyzed consisted of a crude-lipid fraction soluble in alcohol-ether, a protein fraction insoluble in 7 per cent trichloroacetic acid, and a soluble rest-nitrogen.

The nitrogen of the chloroplasts increases with the nitrogen of the remainder of the protoplasm, an approximately constant nitrogen ratio being found to exist between them during the various stages of cell development.

The chloroplasts of tomato and tobacco contain 30 to 40 per cent of the total nitrogen of the leaf-blade material (i.e., exclusive of the midrib and main veins).

The protein of the chloroplast constitutes 80 per cent of the chloroplast nitrogen. The chlorophylls $a + b$ account for another 10 per cent of the nitrogen in the chloroplast.

In the average leaf parenchyma cell it is estimated that 35 to 45 per cent of its protein is contained in the chloroplasts of the cell.

A parenchyma cell, two-thirds of its maximum attainable size, contains about ten times more total-nitrogen and protein-nitrogen than a cell one-third the maximum size. The nitrogen of the fully expanded cell is only 25 per cent greater than the nitrogen of the cell which is expanded to two-thirds its maximum size.

The evidence suggests that the chloroplasts as well

as the non-chloroplast protoplasm possess the ability to synthesize their own proteins.

Assuming a molecular weight of 100,000 for the proteins of the chloroplast, it is estimated that there are approximately thirty chlorophyll molecules for each protein molecule of the chloroplast.

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CONCERNING THE CONDUCTIVE CAPACITY OF THE MINOR VEINS OF FOLIAGE LEAVES¹

Robert B. Wylie

ANY SKELETONIZED dicotyledon foliage leaf reveals the elaborate ramifications of its conductive system. A few major veins dominate the pattern and from their size and distribution appear to be relatively indispensable. By way of contrast the numerous smaller veins seem to serve only limited areas of contiguous mesophyll. However, wounding experiments with living, attached leaves show that many tolerate cutting of some or even all of their primary veins without death of tissue. Such experiments in modified form also demonstrate an unexpected capacity on the part of the minor veins to carry conductive overload and in emergency to serve even remote parts of the blade. They possess qualities not appreciated when viewing the leaf as a whole and are of potential significance, particularly in meeting emergencies. This paper deals with certain experiments illustrating the conductive possibilities of these minor veins.

The average distance between the minor veins in the leaves of 22 species of Iowa deciduous trees and shrubs is 112 μ , measured from xylem to xylem in

the paradermal plane. The border parenchyma sheath surrounding these veins averages 14 μ in radial dimension in the same plane; this leaves the normal width of mesophyll between veins about 85 μ . Each of the ultimate divisions which constitute the plexus of vascular supply in the blade is thus related to a narrow strip of mesophyll less than 50 μ wide on either side of its border parenchyma. This general situation would support the assumption that such members are of only local importance. But their further significance is revealed through response to lesions.

If, in addition to interrupting related major veins, portions of the blade are more or less isolated by incisions, there results further demand upon the lesser veins supplying the distal or isolated portions of the leaf. While tolerance of this type of injury varies greatly with different species, with venation system, and with environing conditions, many leaves show remarkable ability in meeting such abnormal situations, involving necessarily increased conduction through the remaining minor veins of the isthmus connections. While such experiments have been tried with many

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plants, the present paper is concerned only with those relating to lilac (*Syringa vulgaris*). The form used was a dwarf variety, having purplish flowers and leaves of medium size, averaging about 5×7 cm. It was somewhat more tolerant of injury than the larger leaved varieties in this region.

The particular plant, consisting of a cluster of stems 7-9 feet high, stood in a grassy area on sloping ground in full sunlight, except in late afternoon; it was about 35' southeast from two large trees, one a maple and the other an oak; about 50' from two other oaks in the same general direction, while 60-75' to the southwest stood two larger oak trees. Nearer, to the north and east, were a large grapevine, two small *Celtis* trees, and a considerable clump of shrubbery. While this lilac plant was in favorable light relation and had the advantage of deep, clay soil, its area was penetrated by roots of most or all of the nearby trees and shrubs, as well as grasses, involving serious competition for soil water, particularly during the midsummer season.

METHODS.—The leaves were cut by means of a long-jawed paper punch, the upper cutting shell of which had been ground to a keen edge and the lower padded with a piece of cork. With this tool, circular pieces about 4 mm. in diameter could be cut out of the blade. In such way one could excise a segment of a major vein or parts of blade tissue, and by overlapping the cuts there could be removed a zone of blade about 4 mm. wide. Of significance is the fact that in all cases the wounded edges of the leaf were fully exposed to drying and to traumatic water loss. This is important, since short slits cut in a blade give the wounded margins varying degrees of protection, and in no case is there full exposure to the air.

The entire amount of injury to each leaf was completed at one operation on the forenoon of July 20, 1932. They were collected on September 2, and during intervening weeks the plant had only the natural rainfall and received no special attention. If the nature of the wound was such as to greatly weaken the blade, support to distal parts was given by tying stitches across the gap by use of needle and thread. Other than this the wounded leaves were given no aid of any kind. When removed from the plant they were blue-printed and portions of the blade promptly killed and preserved in FAA for histological study. In each type of experiment several leaves were similarly injured. All leaves used were on the southern side of the cluster of stems and at a height of 5-6 feet above the ground. In addition to information as to the conductive capacity of minor veins, it was hoped that microscopic study of the various areas might reveal evidence as to readjustment of tissues to the altered conditions.

The three experiments illustrated in the figures were selected from many involving like principles but with varying width of isthmus and areas of isolated blade. In the simplest case (fig. 1) a peninsula was cut out of the lamina in such a way that the narrow isthmus (3 mm. wide), which did not include any

major or submajor vein, was directed towards the central region of the blade. In the second experiment (fig. 2) a similar peninsula was isolated, but the isthmus, 3 mm. wide, which as before included no large vein, faced outward, making connection near the margin of the blade. It seemed that this latter orientation would offer greater difficulty since water could be secured only through the marginal plexus of minor veins and also because the normal direction of movement of water was reversed in the tissue of this peninsula.

The third experiment (fig. 3) involved much more serious mutilation, since the blade was cut across about 15 mm. from the base, interrupting all major and submajor veins, and leaving a strip of living tissue only 1 mm. wide on the right side, viewed from above, and but 0.8 mm. on the left. The distal, isolated part of the blade measured about 23 sq. cm. At the time of wounding, as the cutting progressed, stitches were introduced by means of a threaded needle, and in this way the outer portion was mechanically anchored, even though this distal part hung downward.

Reference should here be made to local weather conditions during the period of these experiments. The following data are from official government observations in Iowa City, Iowa, noted at a distance of about one mile from this plant. The precipitation for the months of May, June, and July, 1932, was about the average, month by month, for the 5-year period, 1931-1935, inclusive. The month of August had an unusual rainfall (8.84 inches), or about twice the normal precipitation. Half of this fell on two consecutive days, August 1 and 2, and the remainder was in the form of scattered showers near the middle and again near the close of the month. Of greater significance was the daily weather for the period preceding and especially at the time of wounding the leaves on July 20. Official records show no precipitation for the period July 12-21, inclusive, 0.02 inches on the 22nd, and for the remainder of the month only a "trace," on July 26. The experiments then fell early in a period of 21 days of unusual dryness.

For the 8 days preceding the date of wounding the maximum temperature was above 90° each day, and on July 20 (when leaves were injured) reached 97°, with 96° on the following day and 93° on the 22nd. For the five succeeding days the lowest maximum was 88°, with two days at 89°, one at 90°, and another 92°. In brief, the experiments began on the eighth and hottest of a series of dry, clear days, and similar weather with westerly wind continued through the crisis period, which involved the first day following lesion. The 0.02 inch of rain on the 22nd was a local shower, on a day with prevailing west wind; there was no further rainfall in July.

RESULTS.—As noted above, the wounding was carried out on the morning of a hot July day under conditions offering difficult conduction problems due to the nature of the lesions as well as to the temperature and air conditions at the time. Except for

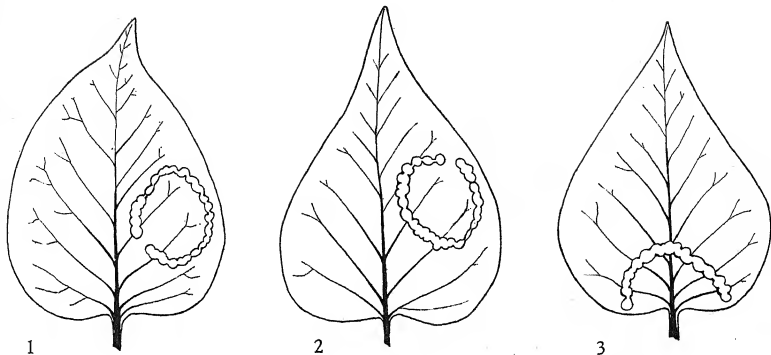


Fig. 1-3.—Fig. 1. Leaf of *Syringa*. $\times 4$. Wounded July 20; collected September 2. Area of peninsula 2.87 sq. cm.; width of living tissue in isthmus, 3 mm. No death of tissue except along wounded margins.—Fig. 2. Leaf of *Syringa*. $\times 4$. Wounded July 20; collected September 2. Area of peninsula 2.8 sq. cm.; width of living tissue in isthmus 3 mm. No death of tissue except along wounded margins.—Fig. 3. Leaf of *Syringa*. $\times 4$. Wounded July 20; collected September 2. Area of distal, isolated portion of blade, 22.95 sq. cm.; width of living tissue of isthmus on right, 1.0 mm.; on left 0.8 mm. No death of tissue except along wounded margins.

the usual drying along all wounded margins, the isolated areas in all three cases remained alive until the time of collection, on September 2, after an interval of 42 days. All conduction during this period was through the narrow, living strips of blade, and in addition to the normal transpiration demands there was traumatic water loss for a time. Careful study of both transverse and paradermal sections from various parts of the blades of all three leaves failed to show any marked changes in any part or cell divisions in any tissue as a result of injury. There were, however, moderate alterations in cell size and shape as well as modified content in certain tissues. Of special interest was the lack of any growth or regeneration of vascular bundles in or near the connecting strips or elsewhere near the lesions.

The third leaf (fig. 3) offered more serious conduction problems, due to the greater distal area and the limited width of connecting strips, so it seemed desirable that its tissues be subjected to more critical analysis. There follow certain comparative measurements of basilar, distal, and marginal parts. Those relating to the connecting strips are difficult of comparison with the broader areas of blade because of their limited width and location at the edges of the leaf. Normal variations in thickness and structure are involved, due to the lateral thinning of the blade. There is also a local thickening along inner (wounded) edges of these strips due to the cicatrice development. Of significance, however, was the lack of any striking modification of the mesophyll even in tissues of these isthmus regions, and notable was the absence of any vascular regeneration.

There was a definite thickening of the distal (isolated) portion of the blade and doubtless also of the

connecting strips. Measurements at stations equally remote from the midrib region, where the blade is appreciably thicker, gave a thickness of 211μ for the distal part and 191μ for the basal area. But variations in normal thickness of the lamina are included in these figures. A survey of a similar uninjured leaf from the same plant gave a contrasting difference in thickness between corresponding areas, the basal being 11.2μ thicker than the distal part. The actual increase in the thickness of the outer part was therefore about 30μ , reversing the normal ratio of thickness for these portions of the leaf.

Turning to the tissues of the blade both upper and lower epidermal layers are thicker in the isthmus zones than elsewhere. The upper epidermis is here about 3μ thicker than over the base and 5μ thicker than on the distal part of the blade. The lower epidermis showed about one half as much increase in thickness over the other areas of lamina. This modification seems to be merely cell enlargement and was not accompanied by division of epidermal cells. The upper palisade cells of the outer part of the blade averaged 2μ greater diameter than those of the basilar portion. These cell diameters were measured at the midpoint, readily identified in either section by the position of the nucleus. The total depth of palisade was at least 12μ more in the outer part than in the base of the blade. The cells of the spongy mesophyll were also somewhat enlarged. This could be determined with greater accuracy in the transverse plane. There was no thickening of the cuticle on the distal part of the blade.

The enlargement of mesophyll cells in the paradermal plane, and especially those of the palisade, was largely at the expense of intercellular space. The

cells were either brought nearer together or more extensively into contact with one another. This operated to cut down the area of mesophyll cell wall exposed against intercellular spaces. A critical analysis of the internal exposed surface in basilar and distal parts was carried out by Dr. Turrell, using the formulae which he developed for such measurements (1936). He found the internal/external surface ratio to be 13.94 for the outer part of this blade and 14.4 for the basal region below the wound. This means that in the isolated area in spite of considerable increase in thickness of blade there was 3.28 per cent less surface of the mesophyll exposed than in the basilar part. It is from this surface that most of the transpiration loss occurs. This reduction doubtless resulted chiefly from the enlargement of palisade cells, tending to make the tissue of that layer more compact, with less surface exposed against intercellular space.

A check on the intervascular interval, or the distance between veins, measured from xylem to xylem in the paradermal sections, showed no significant variations, and the slight differences noted may have been normal and not the result of lesion. The width of the border parenchyma around the veins was very nearly constant in all parts; the average of many counts showed that that of the distal portion of the blade was 0.3μ less than the other parts, and this was probably not due to injury. The average paradermal diameter of minor veins of like rank showed a difference of about one micron in favor of the basilar part; probably a normal difference.

The cells of the distal part of the leaf as well as connecting strips were packed with starch grains. Those of the basal part, collected, of course, at the same time, were practically free from starch. This was due doubtless to inadequate channels for the conduction of sugars from the outer part of the blade. There were undoubtedly other changes in cell content which favored retention of water in this region of the blade.

The behavior of leaves similarly wounded but left on the plant is worthy of record. At the time of autumnal leaf fall, when these wounded leaves as well as others developed abscission layer, the color faded from basilar parts, but the isolated areas continued green. After the leaf fall, they remained conspicuously green for two or three days, lying on the ground, though the bases of these leaves had yellowed and dried. Weighing tests showed, for equal area of blade, that loss of weight during the first 48 hours after leaf-fall was much more rapid from the parts of blade below the injury. The isolated areas had evidently developed a combination of conditions retardative of water loss.

DISCUSSION.—In these experiments total conduction to and from the partly isolated areas of blade was through the narrow isthmus regions. Movement of materials for such distances would be chiefly through the veins, though conduction from cell to cell, important between veins, would have to be taken into

account. Since the epidermal layers are the only continuous tissues in the paradermal plane, it may be that their increased thickness over the connecting strips is related to lateral conduction through these layers. While this could be helpful in a local way, it would be of less importance through greater distance.

There was no obvious modification of the conductive channels of these strips as a result of injury. Simon (1908) found ready regeneration of vascular bundles in wounded herbaceous stems of *Achyranthes* and *Coleus*. Freundlich (1908), working chiefly with leaves of herbaceous plants and cotyledons of seedlings, found vascular regeneration in these organs following lesion. He noted the establishment of new conduction channels which resulted from cell divisions in blade tissues. In the case here under consideration (fig. 3) there was neither increase in size nor in number of veins as a result of injury. In paradermal sections veins could be traced in unaltered condition to the edge of the wound where cicatrization had taken place. There was no modification of the border parenchyma about the veins, nor was there evidence of cell division in any tissue. All demonstrable changes related to alterations in size and content of epidermal and mesophyll cells of the blade.

The maximum demand upon the connecting strips in these experiments was immediately following injury. There was abundant evidence of the later development of numerous devices retardative of water loss by the more or less isolated portion of the blade, but none of these could help during the difficult period on the day of injury. Wounded mid-forenoon of a hot, dry, summer day, there was not only the normal transpiration loss but also evaporation from wounded margins. These traumatic losses were soon reduced by pseudocicatric development and stopped in a few days by cicatric formation (Wylie, 1930). But the wounded leaf probably experienced temporarily a somewhat increased water loss, which, for the distal part, had to be met by the few remaining veins. Any subsequent lessening of demands through slow modifications of the isolated areas of blade could not help in the period of greatest need, at the time of lesion. Even had there been vein regeneration, its value might be discounted (for exposed leaves) because of the necessary delay in its development. With seriously wounded leaves under normal, exposed conditions, the crisis which determines the life or death of portions of the blade is on the day of injury, much earlier than vascular regeneration can occur and before tissue changes can develop in the blade.

Any attempt to evaluate the load carried by the vascular tissue of these connecting strips must be stated in general terms. These lesser veins, which were the only ones involved in these experiments, are not continuous channels but constitute a network which lies between and beyond the larger veins. Since their arrangement is meshlike, with members extending in all directions in the paradermal plane, it is difficult to determine the functional limits of any given segment or unit. One approach would be to

take the average number of veinlets cut across, more or less directly, by transverse sections through the narrow isthmus strips. These components of the meshes might be regarded as governing lengthwise movement of water through the border strips. Certainly all veins at sharp angle to the major directions of flow may be safely left out of account in this connection.

Cuts across the isthmus strips (fig. 3), which had a combined width of 1.8 mm. of living tissue, showed an average of about ten veins. The expected number, recalling the intervacular interval for the blade as a whole, would be somewhat higher. However, due to marginal structural differences, there is wider separation of veins in that region. There is not only a vein-free zone averaging 137 μ along the edge of the blade, but adjacent areas have somewhat wider separation than in the main body of the leaf.

At the time of wounding, all major veins were cut through near the base of the leaf and also 350–500 members of the minor vein plexus, leaving about ten veinlets to supply the distal, isolated portion of the blade. Disregarding, for the moment, the obvious importance of the transected larger veins and thinking only of the minor venation, it is apparent that about 2–3 per cent of the lesser veins was left (in the border strips) to meet the conduction demands. Inclusion of the major veins in this comparison would of course lower the proportion of vascular tissue left intact. While these relative numbers suggest something of the load carried by the remaining veins, such comparison tells only a part of the story, since it does not take into account the total area of leaf blade served by these few veins.

In the uninjured lilac leaf of this variety, the final divisions of the vein system serve each a zone of mesophyll about 80 μ wide, or 40 μ on either side. Near the margins, intervals are somewhat wider, and in these connecting strips, the distance between veins, omitting the border parenchyma, averaged about 140 μ . The length of these service units cannot be determined, due to the mesh arrangement of their members, but in an uninjured leaf, the distribution through major veins would localize in great measure demands upon these ultimate divisions. The area of the outer part of this wounded leaf was 2295 sq. mm., and this was supplied by strips having, in cross section, but 10 veins, representing about 1 per cent of the original vascular supply to this area. This suggests a conductive capacity far in excess of that normally carried by these minor veins. While this load is later reduced by modifications which retard water loss, it is probable that the load was increased immediately following lesion and was, during the crisis period, somewhat greater than the normal demand by the outer part of the blade.

Recalling the mesh-like arrangement of minor veins, attention may be drawn to the significance of this

vein-plexus type of organization which is common to most dicotyledon leaves. Beyond the larger veins and between their chief divisions there is spread out in the blade a network of lesser channels so intimately united that they can meet demands from any direction and so equalize the flow to all parts of the leaf. This plexus system in an uninjured leaf takes care of the distribution of material to, and collection from, the real working units, which are the narrow strips of mesophyll lying along minor veins. Apparently, the members of this plexus possess also an unrealized capacity for carrying overload to meet emergencies.

Because the leaf as a photosynthetic organ cannot be adequately protected and at the same time expose tissues to light, it has been peculiarly subject to injury at all stages of its development. Traumatic tolerance has been intimately related on one hand to prompt cicatrization of wounds and on the other to a capacity for handling conductive problems induced by lesions. Since all leaves are peculiarly subject to injury, natural selection has conserved types of foliar organization having ready capacity for pseudocicatricial and cicatricial formation and possessed of a minor vein organization adapted to handle overload in any direction.

SUMMARY

Leaves of a lilac (*Syringa vulgaris*) were wounded in various ways involving the interruption of major veins and the partial isolation of portions of blade, leaving isthmus connections which contained only minor veins.

The few remaining small veins of the connecting strips sustained the distal areas for six weeks and demonstrated a conductive capacity in excess of that required of them in the normal, uninjured leaf.

There was neither enlargement of veins following lesion nor regeneration of vascular tissue in the connecting strips of blade.

Tissue changes in the connecting strips and isolated areas consisted only in the enlargement of epidermal and mesophyll cells and in altered cell contents.

In the most extreme case (fig. 3) the distal area, though considerably thickened, exposed 3.28 per cent less mesophyll surface against intercellular space than did the basilar part of the same leaf.

Isolated areas of blade were appreciably thickened and contained much starch at the end of the experiment.

In the evolution of the foliage leaf capacity for prompt cicatrization of wounds and the possession of a minor venation capable of conductive overload in any direction have been factors of value in dealing with the inevitable problems of traumatism.

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KALANCHOE: THE GENUS AND ITS CHROMOSOMES¹

J. T. Baldwin, Jr.

THE GENUS.—*Kalanchoe*—"from the Chinese term for one of the species" (Harvey, 1894)—is a genus of the Crassulaceae. It is separable from other members of the family in having a 4-parted flower and a tubular corolla with which eight stamens in two circles are fused. *Bryophyllum* and *Kitchingia* likewise have these characters but are considered here to be invalid genera; critical study has revealed them to intergrade with *Kalanchoe*. Hamet (1907, 1908, 1915, 1916), the only monographer of this complex of the Crassulaceae (designated subfamily Kalanchoideae by Berger, 1930), reduced *Bryophyllum* and *Kitchingia* to *Kalanchoe*. Perrier (1923, 1924, 1928), and earlier as a collaborator with Hamet (Hamet and Perrier, 1912, 1914, 1915), is the other worker most familiar with the group; he concurs with Hamet's treatment.

Adanson, in 1763, published *Kalanchoe*, later sometimes written *Calanchoe*. Salisbury, in 1806, established *Bryophyllum* and assigned the name *B. calycinum* to a plant with an inflated calyx; that plant apparently already had a varied nomenclatorial history of two hundred years, extending from *Sedum Madagascariense* of Clusius (1605) to *Calanchoe pinnata* of Persoon (1805). Different botanists (DeCandolle, 1828; Dalzell, 1852; Hance, 1873) have questioned the generic standing of *Bryophyllum*, and Hamet (1907) reported *K. pinnata* (*Bryophyllum calycinum*) and *K. laciniata* to be the extremes in a long series of intermediate species which could not be segregated precisely into genera, but *Bryophyllum* persists in systematic literature as a "good" genus. For example: Berger (1930) retains the genus and recognizes for it about twenty species. Similarly, Baker (1881) founded *Kitchingia*; Baillon (1885) reduced the genus to *Kalanchoe*. Baker (1887) accorded with this rejection of his genus, but many subsequent writers have kept it.

Evidence of another sort makes suspect the status of *Bryophyllum* and *Kitchingia* as entities distinct from *Kalanchoe*. As mentioned above, Berger (1930)

placed these three genera in the subfamily Kalanchoideae. Mauritzon (1933) found this subfamily to be well distinguished embryologically from the other five. Proner (1934) was able to separate it from the others by means of characteristic idoblasts occurring in the stems and leaves of Crassulaceae. Though a number of species have been transferred to this *Kalanchoe*-aggregate, no species seems ever to have been removed from it. The subfamily appears to be a natural division. To associate a representative of the group with its congeners is not difficult, but to refer that representative to one of three genera in the group creates taxonomic trouble. As a consequence, changes in specific status have been of high frequency, and reduction has been as greatly extragenetic as intergeneric (*Index Kewensis*). Were the genera distinct, this situation would not be expected. And, finally, data from chromosome studies reveal that the Kalanchoideae are a group of close phyletic relations and that this subfamily is the expression of cytologically recognizable trends in the evolution of the family; these data do not afford sufficient basis for drawing the generic lines that have been proposed.

An estimate based on Hamet's concept of the group, and taking into account later discoveries, reckons 125 to be the approximate number of valid species described for *Kalanchoe*. About half of these species are restricted to Madagascar. Most of the others are African. There are, however, a few species in southern and eastern Asia, several in Africa and Socotra, or in Socotra alone, and two species (*K. pinnata* and *K. laciniata*) occurring in nearly all the tropical regions of the world.

Kalanchoes are fairly popular, particularly as Christmas plants, and seem destined to become more so (Rowntree, 1936). They are easy to grow, one of them (*K. Daigremontiana*) being called "the easiest plant in the world to propagate" (Swingle, 1934). The most valuable horticultural representative of the group is *K. Blossfeldiana* (*K. coccinea* of the trade); "it may be grown readily from seed and if the seed is sown during January to March, the plants will begin to flower after one year, thus at a time when showy pot plants are scarce. . . . An additional advantage is that the flowers of any one inflorescence after full development remain fresh for a period of 7-8 weeks" (Schweickerdt, 1936). Other species, too, will doubtless come equally well from seed, and the seeds are numerous. The flowering period for other

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species is also long, and many species bloom at the same time: twelve species were in flower in the greenhouses of Cornell University during the first week of 1938. The species exhibit a wide range of characters, though the plants are much alike in their chromosomal make-up. *Kalanchoe* appears, therefore, to be an excellent group to hybridize inter-specifically. The flowers would offer no technical difficulty in breeding experiments. Valuable hybrids could be preserved by vegetative means. The genus, no doubt, has a future of real horticultural interest.

The cultivated plant index maintained at the Bailey Hortorium shows that kalanehoes are in the European trade under fifty-three names and in the American trade under forty-four names, a total of seventy-one different designations: *Kalanchoe*, sixty; *Bryophyllum*, eight; and *Kitchingia*, three. Many of these names have systematic standing; others do not. Often a number of names is applied to the same plant, sometimes all of them erroneously. An artificial key to some of the species cultivated in the United States is included here. The key covers only those species represented in the herbarium of the Bailey Hortorium, but they appear to be most of the species now in the American trade. The key is so constructed that a few species which seldom flower may also be identified.

KEY TO THE SPECIES

- A. Stem leaves glabrous B.
- B. Inflorescence axillary, compact, hairy, borne on peduncle (with three pairs of bracts) up to 10 in. long; axillary stolons present, up to 12 in. long, leafed at tip; leaves 8-10 pairs, crowded, suborbiculate-oblong, obtuse-subobtusate, tapering at base into large, stem-clasping petioles; calyx not exceeding 5 mm., lobes acute, shorter than tube; corolla up to 12 mm., lobes pale lilac to rose:
K. synsepala
- B. Inflorescence not borne on long axillary peduncle C.
- C. Flowers 1-3, minutely hairy; stems creeping; calyx deeply lobed, lobes broad, ovate; corolla inflated, bright red:
K. uniflora
- C. Flowers many; stem erect or climbing D.
- D. Sepals fused at base into a conspicuous tube; flowers more or less pendent ... E.
- E. Carpels diverging from the base; leaves peltate; leaf-scars not joined; petiole affixed about 1 cm. above rounded leaf-base; leaves crenulate; calyx small, lobes about as long as tube; corolla up to 27 mm., pale pink to rose; stem erect, up to 7 ft.:
K. peltata
- E. Carpels converging; leaves never peltate, occasionally subpeltate F.
- F. Stem climbing, up to 10 ft.; leaves sessile, lanceolate-linear, stem-clasping; corolla campanulate, purplish green to dark violet:
K. scandens

F. Stem erect, not climbing G.

G. Leaves sessile, whorled or alternate, subcylindric, toothed at apex; flowers salmon to scarlet:
K. verticillata

G. Leaves petiolate, opposite, flat. H.

H. Leaf-scars joined I.

I. Petiole very distinct, often equalling leaf-blade in length; blade up to 2 in., not mealy, sinuate-crenate, ovate-lanceolate, occasionally subpeltate; calyx less than 1 cm.; corolla pink to red; stem up to 3 ft.:
K. miniata

I. Petiole not very distinct, not more than $\frac{1}{2}$ length of leaf-blade; blade up to 8 in., mealy, crenulate, ovate-lanceolate, never subpeltate; calyx exceeding 2 cm.; corolla greenish yellow to pale red; stem up to 2 ft.:

K. Gastonis-Bonnieri

H. Leaf-scars not joined J.

J. Corolla contracted at base.

K.

K. Calyx not exceeding 12 mm.; leaves simple, undulate to dentate, ovate-obovate, blue-green to gray-green; flowers salmon to orange:

K. Fedtschenkoii

K. Calyx exceeding 14 mm.

L.

L. Leaves simple, obovate, cuneate at base, sinuate, green to purple; flowers salmon pink to rose:
K. Waldheimi

L. Leaves simple to pinnate; blades of leaves and of leaflets oblong-elliptical, crenate, green; flowers reddish (often greenish-yellow on herbarium specimens):

K. pinnata

J. Corolla not contracted at base M.

M. Flowers reddish; calyx inflated; leaves crenate, elliptical, usually auricled:

K. laxiflora

M. Flowers purplish or lavender; calyx not inflated; leaves dentate,

lanceolate, never auricled, some of them occasionally subpeltate:

K. Daigremontiana

- D. Sepals almost free; flowers more or less erect

- N. Corolla tube exceeding 2 in., white:

K. marmorata

- N. Corolla tube not exceeding 1 in., not white

- O. Corolla red; leaves entire or crenulate

- P. Corolla twisted in bud and in old flowers; stem sparsely branched, up to 4 ft.; leaves roundish-obovate, oblanceolate or spatulate; inflorescence not very many-flowered:

K. rotundifolia

- P. Corolla not twisted; stem much branched, up to 1-1/2 ft.; leaves oblong or ovate-oblong; inflorescence dense, very many-flowered:

K. Blossfeldiana

- O. Corolla yellow to reddish orange ..

- Q. Stem quadrangular; leaves sessile, cuneate; calyx lobes not exceeding 3 mm.; corolla tube 14-17 mm., yellow:

K. longiflora

- Q. Stem round; leaves clearly petiolate; calyx lobes usually exceeding 3 mm.; corolla tube less than 14 mm.

- R. Leaves coarsely crenate; plant glabrous throughout; flowers yellow; stem up to 6 ft.:

K. crenata

- R. Leaves entire, sinuate, or parted; plant glabrous throughout or hairy in upper part; flowers yellow to reddish orange; stem up to 4 ft.:

K. laciniata

- A. Stem leaves not glabrous

- S. Vested with scales

- T. Corolla urceolate, yellow, exceeding 7 mm.; calyx lobes longer than broad, reflexed with age; leaves not crowded, ovate to ovate-lanceolate, acute; stem up to 5 ft.:

K. orgyalis

- T. Corolla tubular, white to greenish white, not exceeding 6 mm.; calyx lobes broader than long, applied against the corolla; leaves crowded, ovate to suborbiculate, very obtuse; stem up to 20 ft.:

K. Hildebrandtii

- S. Vested with hairs

- U. Hairs stellate

- V. Leaves sessile, alternate, obtuse, tapering at base, usually crenulate in upper part, occasionally entire; calyx not exceeding 6 mm., lobes more or less obtuse;

corolla tube exceeding 1 cm., yellow, much longer than lobes which are broader than long, violet; stem up to 3-1/2 ft.:

K. tomentosa

- V. Leaves petiolate, opposite, usually peltate, acute to subacute, hastate, irregularly dentate; calyx exceeding 6 mm., lobes acute, mucronate; corolla tube less than 1 cm., greenish yellow, usually longer than lobes, occasionally shorter; corolla lobes longer than broad, greenish yellow with violet lines; stem up to 20 ft.:

K. behavensis

- U. Hairs single

- W. Plant glandular, aromatic; corolla lobes reflexed, purple; corolla tube greenish yellow; leaves oblong to lanceolate, acute; stem laxly erect, up to 2 ft.:

K. aromatica

- W. Plant not aromatic; corolla lobes not reflexed, colored like corolla tube, yellow to reddish orange; stem exceeding 2 ft.

- X. Corolla tube up to 1 in.; calyx lobes deltoid; flowers pendent on pedicels exceeding 4 mm.; leaves oblong-elliptical, coarsely crenate:

K. hirta

- X. Corolla tube not exceeding 1/2 in.; flowers not pendent, sessile or with pedicels less than 4 mm.

- Y. Flowers sessile, crowded; calyx lobes linear-oblong:

K. velutina

- Y. Flowers not sessile, not crowded; calyx lobes linear to deltoid:

K. laciniata

CHROMOSOMES OF THE GENUS.—Chromosome counts have been made for about a fourth of the *Kalanchoe* species. The investigated species, twenty-six identified and seven unidentified, are listed alphabetically for each of the chromosome numbers found. Six of the species were examined prior to the present study (Taylor, 1926; Skovsted, 1934; Toyohuku, 1935; Sugiyama, 1936); three of them have been reinvestigated. *K. Daigremontiana* is biennial or triennial; the other species studied are perennial. A few annual species occur in the genus.

Iron-aceto-carminic smears of anthers, root-tip smears made by a modification ² of Warmke's (1935) method, and crystal-violet preparations of sectioned roots were used in the present work. Most of the plants came from the United States Division of Plant Industry. Specimens of *K. pinnata* and *K. laciniata* were kindly sent by Dr. N. B. Mendiola from the

² The modification is: kill the material for 5 minutes to 12 hours in Carnoy's solution (3 parts chloroform, 2 parts absolute alcohol, and 1 part glacial acetic acid); transfer for 2 or 3 minutes to a solution of 1 part 95 per cent alcohol and 1 part concentrated hydrochloric acid; place the material back into the Carnoy's solution for at least 5 minutes, and smear in iron aceto-carminic.

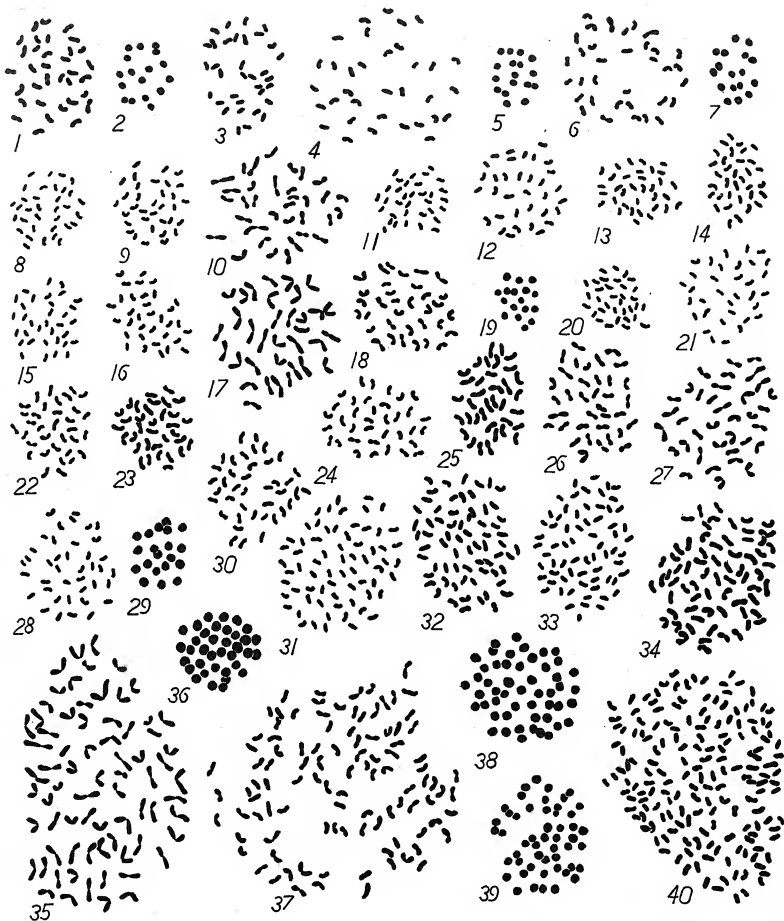


Fig. 1-40. Chromosomes of *Kalanchoe*.—Fig. 2, 5, and 7: $n=17$ at first metaphase.—Fig. 29: $n=20$ at first metaphase.—Fig. 36: $n=36$ at second metaphase.—Fig. 38 and 39: $n=51$ at first metaphase.—The remaining figures are of somatic metaphase: Through fig. 23, $2n=34$; fig. 24-27, $2n=36$; fig. 28 and 30, $2n=40$; fig. 31-34, $2n=68$; fig. 35, $2n=72$; fig. 37, $2n=102$; and fig. 40, $2n=ca. 170$.—Fig. 8, 9, 11-16, 18, 21, 22, 24, 28, and 31 were drawn from crystal violet preparations, the others from smears. Fig. 2, 3, 5, 7, 19, 20, 23, 25, 26, 29, 32, 34, 36, 38, and 39 were drawn $\times 3700$, the others $\times 4900$, and reduced respectively to $\times 2220$ and $\times 2940$.

Philippine Islands. Specimens of most of the species studied are in the herbarium of the Bailey Hortorium.

The chromosomes are small throughout the genus. For this reason, although morphological differences are often comparatively great within a specific complement, such characteristics were not constantly recognized. In the rather limited number of meiotic divisions examined for the genus, no irregularities were observed. The species studied with n -numbers of 17 are free from multivalent association, and secondary association, if present, is not close. In some of the other species, however, approximation of bivalents is such that the association may sometimes be primary rather than secondary.

K. Aliciae Hamet = *Bryophyllum Aliciae* (Hamet) Berger. Madagascar. $n = 17$ (Skovsted).

K. aromatica Perrier. Madagascar. $2n = 34$, $n = 17$ (Plant Introduction No. 101362, fig. 1, 2).

K. Blossfeldiana v. Poellnitz = *K. globulifera* Perrier var. *coccinea* Perrier. Madagascar. $2n = 34$ (plants in Cornell greenhouses, fig. 3).

K. Daigremontiana Hamet and Perrier = *Bryophyllum Daigremontianum* (Hamet and Perrier) Berger. Madagascar. $2n = 34$ (P. I. 78434, fig. 4), $n = 17$ (H424, Cornell greenhouses, fig. 5).

K. Fedtschenkoi Hamet and Perrier. Madagascar. $2n = 34$ (P. I. 78427, fig. 6, and P. I. 101364), $n = 17$ (P. I. 78427, fig. 7).

K. Gastonis-Bonniere Hamet and Perrier. Madagascar. $2n = 34$ (P. I. 78428, fig. 8).

K. globulifera Perrier. $n = 17$ (Sugiuira).

K. laxiflora Baker = *Bryophyllum crenatum* Baker and *K. crenata* Hamet. Madagascar. $2n = 34$ (P. I. 77909, fig. 9).

K. longiflora Schlechter. Africa. $2n = 34$ (P. I. 86234, fig. 10, and plants in Cornell greenhouses).

K. marmorata Baker. Africa. $2n = 34$ (P. I. ? , fig. 11).

K. miniata Hilsembach and Bojer = *Kitchingia miniata* (Hilsembach and Bojer) Baker and *Bryophyllum miniatum* (Hilsembach and Bojer) Berger. Madagascar. $2n = 34$ (P. I. 78429, fig. 12): a subpeltate race (*K. subpeltata* Baker) also has 34 somatic chromosomes (P. I. 101359, fig. 13).

K. peltata (Baker) Bailon = *Kitchingia peltata* Baker. Madagascar. $2n = 34$ (P. I. 78437, fig. 14).

K. prolifera (Bowie) Hamet = *Bryophyllum proliferum* Bowie. Madagascar. $2n = 34$ (Toyohuku).

K. rotundifolia Haworth. Africa and Socotra. $2n = 34$ (P. I. 97616, fig. 15).

K. scandens Perrier = *Bryophyllum scandens* (Perrier) Berger. $2n = 34$ (P. I. 101360, fig. 16).

K. tomentosa Baker. Madagascar. $2n = 34$ (P. I. 78430, fig. 17).

K. velutina Welwitsch. Africa. $2n = 34$, $n = 17$ (P. I. 91991, fig. 18, 19).

K. Waldheimi Hamet and Perrier. Madagascar. $2n = 34$ (P. I. 101372, fig. 20).

Kalanchoe sp. $2n = 34$ (P. I. 78433, fig. 21).

Kalanchoe sp. $2n = 34$ (P. I. 79479, fig. 22).

Kalanchoe sp. $2n = 34$ (P. I. 86826, fig. 23).

K. beharensis Drake. Madagascar. $2n = 36$ (P. I. 91525, fig. 24). An atypical plant with deeply notched leaves also has 36 somatic chromosomes (P. I. 91525, fig. 25). This species is available in the American trade as *Kitchingia mandrakensis* ($2n = 36$) and as *Kitchingia peralta* ($2n = 36$).

K. Hildebrandtii Baillon. Madagascar. $2n = 36$ (P. I. 101367, fig. 26).

Kalanchoe sp. $2n = 36$ (P. I. 91526, fig. 27).

K. pinnata (Lamarck) Persoon = *Bryophyllum pinnatum* Kurz and *Bryophyllum calycinum* Salisbury. Tropics of the world. $2n = 40$ (38?) (Taylor); $2n = 40$ (Toyohuku); $2n = 40$ (273, plants from the Philippine Islands, fig. 28), $n = 20$ (plants in Cornell greenhouses, fig. 29). Association among some of the elements at first and second metaphase is such that often only 18 or 19 units can be counted. This apparent attraction between bivalents possibly indicates a recent origin of this gametic number which is unusual in the genus. But this species is widespread and is thus to be considered old; some of the forms described for the species may have an n -number less than 20.

K. uniflora (Stapf) Hamet = *Kitchingia uniflora* Stapf and *Bryophyllum uniflorum* (Stapf) Berger. Madagascar. $2n = 40$ (P. I. ? , fig. 30).

K. laciniata (Linnaeus) DeCandolle. This species as conceived by Hamet is extremely polymorphic and occurs in about all the tropical and many of the sub-tropical regions of the world. Various plants given a different specific designation by other workers are encompassed within this concept. The group needs taxonomic revision. Hamet's treatment is followed here. $2n = 34$ (*K. aegyptiaca*, Skovsted); $2n = 68$ (274, a Philippine plant approaching *K. varians* Haworth, fig. 31); $2n = 68$ (P. I. 103502, *K. spatulata*, fig. 32; a plant of this identity also had $2n = 34$).

K. verticillata Elliott = *K. tubiflora* Hamet. Madagascar. Berger (1930) referred this plant to *Bryophyllum tubiflorum* Harvey, an African species considered by Hamet (1908) to be synonymous with *K. delagoensis* Ecklon and Zeyher. $2n = 68$ (P. I. 101361, fig. 33).

Kalanchoe sp. $2n = 68$ (P. I. 78485, fig. 34).

K. synsepala Baker. Madagascar. $2n = 72$, $n = 36$ (P. I. 78431, fig. 35, 36). It is difficult to count the meiotic chromosomes because of their close association.

K. crenata Haworth. Tropical Africa. $n = ca. 51$ (Skovsted); $2n = 102$ (P. I. 86824, fig. 37), $n = 51$ (P. I. 81171, fig. 38; P. I. 77909, fig. 39). The species is a high polyploid of 17, but there is no pronounced secondary grouping of meiotic chromosomes.

Kalanchoe sp. $2n = ca. 170$ (P. I. 97614, fig. 40).

Kalanchoe sp. $2n = ca. 500$ (P. I. 103601). This number is an estimate, not a count, but this approximate number of chromosomes was present in many somatic metaphase plates examined. It is not, therefore, to be considered that the estimate is founded on cells with chromosomes characteristically becoming somatically doubled, as is, well known, for example,

in *Spinacia* (Vid. Lorz, 1937), or on cells with the chromosome number abnormally increased, as in *Crepis tectorum*, a plant with a normal somatic number of 16 having in a root-tip cell more than 500 chromosomes (Nawaschin, 1926). It is apparent that in this *Kalanchoe* it could not be determined whether or not the number differed somewhat from cell to cell; such variation would not be surprising; Christoff and Papasova (1937) in a single root of *Petunia* with a normal number of 28 found all numbers from 28 to 33; Simonet (1929) had made similar observations on *Linum*. The only cutting of this *Kalanchoe* produced very few roots; the condition is unusual for the family. It is hoped that additional material may be available for physiological and further cytological study.

Each of the chromosome numbers precisely determined for *Kalanchoe* is divisible by 17, 18, or 20, and, of these "basic numbers," 17 is taken to be primary. Frequency of occurrence affords not the only reason for this inference; the species with the derived (secondary) numbers are specialized in some significant regard. *K. beharensis* and *K. Hildebrandtii*, each with 36 somatic chromosomes, are among the woodiest of the Crassulaceae; the outstanding characteristic of the family is its fleshy habit. In this connection, it is interesting that *Sedum populifolium* is the least herbaceous species in Section Telephium and has an n -number of 11 which is inferred to be secondary for the section (Baldwin, 1937). Also, the more or less shrubby Mexican species of *Sedum* belong to a highly evolved chromosomal system (Unpub.). *K. beharensis* and *K. Hildebrandtii* will attain heights of twenty feet (Hamet and Perrier, 1914; Swingle, 1931); probably no other members of the family grow nearly so tall. *K. synsepala* ($2n = 72$) is exceptional in its axillary peduncles and stolons. *K. uniflora* ($2n = 40$) is unusual in its creeping habit and few flowers. *K. pinnata* ($2n = 40$) has specialized calyx characters. In mentioning certain exceptional characters for these species with secondary numbers of chromosomes, it is not implied that species with primary numbers do not likewise exhibit modifications of striking sorts; it does appear significant, however, that the species here considered to be chromosomally secondary are also morphologically specialized. It is of significance also that meiotic association of bivalents was observed to be more marked in the species with 20, 36, and 51 chromosomes than in those with 17. Though the 18- and 20-chromosomal systems presumably deviated from a primary trend of 17, obviously certain species belonging to these derived tendencies may be more ancient than many species of the primitive 17-system. Thus, the wide distribution of *K. pinnata* may be attributed to an early divergence of the species from the main developmental trend of the genus.

Nine of the species studied have been referred, at one time or another, by systematists to *Bryophyllum*, and two of the nine and another species to *Kitchingia*. These generic segregations have not coincided with

the several chromosome series in the group. None of the species in the 18-system is among those ten, but the two in the 20-system are. The fact is of interest. These two species are phylogenetic extremes. They and others unequivocally within *Kalanchoe* form a morphological continuum. All these species, therefore, seem properly to be of this genus. Certain workers will object to this concept, and naturally; taxonomic categories in intergrading complexes are founded to a large extent on opinion. The evidence from chromosome morphology is of little taxonomic aid in a group with chromosome size, shape, and constrictions as uniform as they are in *Kalanchoe*. But chromosome number and behavior allow a recognition of trends in the evolution of such a group. These trends are fundamental; the taxonomic categories with names and ranks may or may not be.

To distinguish generic limits in the Crassulaceae is difficult. The taxonomic problem is paralleled by karyological complexity. Chromosome numbers have been determined for about one hundred fifty species of the family. Those numbers have a $2n$ -range of 8-ca. 500, and the range comprises about forty different numbers in many cytological trends. In *Sedum* alone there are at least a dozen of these lines of chromosomal evolution. They have points of interfusion. It is not surprising that the taxonomy of the family is difficult. The Crassulaceae are a dynamic group with "an intricate fabric of lines of descent" (Cook, 1907).

Crassuloideae and Cotyledonoideae are the other subfamilies at all well represented in the geographic area of Kalanchoideae. The basic numbers for the first of these subfamilies are 8 and 7 (Baldwin, 1936), and the n -number of the only chromosomally known species of the second is 9 (Unpub.).³ The primary basic number, 17, of Kalanchoideae (*Kalanchoe*) is the sum of 8 and 9, thus suggesting hybridization of members of two subfamilies with subsequent amphidiploidy as the mode of origin of Kalanchoideae. If chromosome size is controlled by the genotype (Darlinton, 1932; Thomas, 1936), it is not surprising that the chromosomes in *Kalanchoe* are much smaller than in the complexes postulated to be its parental strains. The chromosome number relations of Kalanchoideae parallel those of Pomoideae (Sax, 1931); Pomoideae might have originated from amphidiploid hybrids between two other subfamilies of Rosaceae (Anderson and Sax, 1935). At any rate, from a family standpoint, all the numbers known for *Kalanchoe* are apparently polyploid, for every gametic number from 4 to 13 has been determined for the Crassulaceae, and the various data indicate that 4 is the initial number of the family (Unpub.). That none of the meiotically investigated *Kalanchoe* species with 17 as a gametic number showed an association of multivalents gives some indication that the genus is allopolyploid. The weight of this evidence is less

³ Toyohuku (1935) reported 12 as the gametic number of two *Cotyledon* species, but the systematists have transferred those species to *Sedum*.

sened by the fact that no multivalents were observed in those species (euploid and aneuploid) presumably derived from species with 34 somatic chromosomes. But absence of multivalents is theoretically to be expected in an autopolyploid that has been long in existence; the chromosomes would become differentiated into pairs associating as bivalents. Attainment of bivalent individuality in autopolyploids with chromosomes of a low chiasma frequency would, inferentially, result from few changes, and chromosomes originally similar would continue to be much alike, affinity being indicated through secondary association.

By regeneration experiments on *Bryum* a polyploid race with low fertility was produced; in the course of eleven years fertility became quite normal (Wettstein, 1938). This relation between the degree of fertility and the passage of time is highly significant and has an obvious bearing on the general discussion given here. The experimental results hold fundamental implications for a family so chromosomally variable as the Crassulaceae and for one so well adapted for non-sexual reproduction.

SUMMARY

Evidence from taxonomy, embryology, and cytology indicates that the Kalanchoideae are a well-defined group in which relationships are intimate. Within this complex it is difficult to make morphologically precise segregations on a generic level. It seems proper, therefore, to follow those systematists who reduce *Bryophyllum* and *Kitchingia* to *Kalanchoe* and thus to recognize a single genus for the subfamily.

Kalanchoe, as considered here, includes about 125 species; chromosome numbers have been determined

for approximately a fourth of them. The chromosomes are small throughout the genus; their characters are of slight taxonomic value. The numbers determined are inferred to belong to a primary system of 17 and secondary systems of 18 and 20. Reasons for this inference are several: preponderance of $2n$ -numbers divisible by 17, specialized characters in the species presumably derived from the 17-trend, and a more close (probably secondary) association of bivalents in species with gametic numbers of 20, 36, and 51 than in those with 17. Polyploid forms were found in the 17- and 18-systems. The generic lines that have been proposed for this *Kalanchoe*-aggregate do not coincide with these trends in the chromosomal development of the group.

A $2n$ -number of ca. 500 is estimated for one of the species. Cuttings of this species produce roots sparingly; the situation is unusual for the family.

The initial basic number for the Crassulaceae is deduced to be 4. Many interfusing chromosomal trends are distinguishable in the family. The taxonomic complexity of the group thus has a karyological parallel. It is implied that the ability of the family to propagate itself so well by non-sexual means has been an important factor in the attainment of its present cytological state.

The suggestion is offered that *Kalanchoe* might have originated from a hybrid of species representing two other subfamilies with subsequent amphidiploidy. A similar suggestion has been made for the origin of Pomoideae.

A taxonomic key to some of the kalanchoes in the American trade is included.

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STUDIES IN THE GENUS LABORDIA GAUD., WITH A NEW VARIETY IN MEGALODONTA E. L. GREENE¹

Earl Edward Sherff

IN THE COURSE of a monographic study of the genus *Labordia* Gaud., of the Hawaiian Islands, I have found various new species and varieties, most of which are set forth in the following pages.² To these is added a hitherto undescribed variety of *Megalodonta Beckii* (Torr.) Greene.

Labordia Wawrana sp. nov. — Rami brunneo-grisei, subteretes, nodis saepe numerosis, stipulis distinctis. Folia ad et prope apices ramulorum disposita, oblongo-oblancoolata, apice subabrupte acuminata, magna pro parte sensim in petiolum marginatum hispidulum plerumque sub 1 cm. longum angustata, membranacea, supra glabra, infra hispida, 1-2 dm. longa et 3-6 cm. lata, venis lateralibus principalibus utroque latere ≈ 20 . Inflorescentia cymosa 15-30-flora, perspicue hispida, cernua. Flores (tantum masculini visi) tenues. Calyx hispidus, profunde saepe usque ad basin sessilis, 10-12 mm. longus, lobis lineari-subulatis. Corolla extus breviter intus longe albo-hispida, tubo 11-16 mm. longo et plerumque 2-3.5 mm. crasso, lobis lineari-subulatis 8-9.5 mm. longis. Antherae oblongo-lineares, circ. 2.5-2.7 mm. longae. Pistillodium circ. 13-14 mm. longum; stylo tenui dense nisi ad summam patentis-setoso, supra

sepala in stigma angustissime obconicum circ. 4.5 mm. & longum producta.

Specimens examined: ³ *Dr. Heinrich Wawra* 2200b, Waialeale, Isl. Kauai, between end of December, 1869, and May 1, 1870 (2 type sheets, Mus. V.).

Easily distinguished from other species of *Labordia* on Kauai by its large leaves.

Labordia venosa sp. nov. — Fruticosa forsitan arborescens; ramulis subteretibus, brunneo-griseis, apicem versus (per 1-4 internodia) antrorsum arcuataque adpresso-setulosi, alibi glabris. Folia tenuiter petiolata petiolis submarginatis, hispidulis, 0.8-1.5 cm. longis; lamina obovata vel obovata-oblonga, marginibus subrepando-integra, basi sensim apice plus minusve abrupte angustata, 6-10 cm. longa et 2-4.5 cm. lata, perspicue venosa, supra coriacea et sub-

³ The following abbreviations are used for the depositories of specimens cited in this paper: Berl., Berlin Botanical Garden; Bish., Bernice Pauahi Bishop Museum, Honolulu; Brit., British Museum of Natural History, London; Calif., University of California Herbarium; Corn., Cornell University Herbarium; Deg., herbarium of Otto Degener, Honolulu; Del., Delessert Herbarium, Geneva; Field, Field Museum of Natural History, Chicago; Kew, Royal Botanical Gardens, Kew; Gray, Gray Herbarium of Harvard University; Minn., University of Minnesota Herbarium; Mo., Missouri Botanical Garden, St. Louis; Mun., Munich Botanical Garden; Mus. V., Museum of Natural History, Vienna; N. Y., New York Botanical Garden; Par., Museum of Natural History, Paris; U. S., United States National Museum, Washington.

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² An additional species, from the Island of Kauai, has been named for one of its discoverers, Mr. Otto Degener, and has been published in a recent issue of Mr. Degener's New Illustrated Flora of The Hawaiian Islands (fam. 302, Aug. 24, 1938).

glabra, infra secundum venas venulasque perspicue hispida aliter glabra. Stipulae auriculoideae, intrapetiolares, manifestae, 2-4 mm. altae. Inflorescentia terminalis, sessilis, compacta, ad anthesin dimissa 3-4 cm., cum fructibus diametro 4-6 cm., 10-30-flora, pedicellis ultimis perspicue hispidis ad anthesin \pm 2 mm. sed demum (cum fructibus) usque ad 1 (raro 1.5) cm. longis. Calyx inferne pubescens, superne glaber, 4-6 cm. longus, profunde (fere usque ad basin) 5-lobatus; lobis linearilanceolatis vel linearilanceolatis, 1-2 mm. latis, apice obtusis vel subacutis. Corolla circ. 1 cm. longa, extus sparsissime pubescens, 5-lobata; lobis linearibus, acutis, 4-5 mm. longis; tubo usque ad 3.5 mm. crasso. Capsula atra, 2-mera, (1-) 1.2-1.6 (raro -1.8) cm. longa, valvis extus plus minusve puberulis apicem versus perspicue carinatis.

Specimens examined: *Otto Degener* 10267, rain-forest, Pipe-Line Trail, Olinda, East Maui, June 26, 1927 (Field); *Degener* 10268, same place, June 20, 1927 (Deg.; Field); *Degener* 10270, same place, June 27, 1927 (Berl.; Deg.; Field); *Degener & D. LeRoy Topping* 10263, same place, Jul. 30, 1927 (Deg.; Field); *Charles N. Forbes*, Keanae Gap, Haleakala Crater, East Maui, Aug. 7, 1919 (Bish.); *Forbes* 152-M, woods near Ukulele, above Olinda, July, 1910 (Bish.); *Forbes* 637-M, Keanae Pali, east of Ukulele, Jul. 17, 1919 (Bish.); *Forbes* 760-M, Ukulele, July, 1919 (Bish.); *Forbes* 762-M, same place and date (Bish., fruiting material, 2nd type sheet); *Forbes* 882-M, east of Ukulele, Jul. 20, 1919 (Bish.); *Forbes* 888-M, same place, Jul. 21-23, 1919 (Bish.); *Forbes* 963-M, Ukulele, Jul. 27, 1919 (Bish.; Field); *Forbes* 1237-M, north slope of Haleakala, East Maui, Aug. 23, 1919 (Bish.; Field); *Forbes* 1253-M, Ukulele, Aug. 4, 1919 (Berl.; Bish.; Field; Gray); *Joseph F. Rock* 8538, Honomanu, East Maui, September, 1910 (Bish.); *Rock* 8627 (Bish., flowering material, 1st type sheet; Mus. V.).

The specimens studied had been determined as *L. Grayana* Hillebr., which reduces to a variety of *L. Hedyosmifolia* Baill. From that species and its varieties it differs in numerous characters of foliage, flowers, and fruits, but can be distinguished perhaps most easily by the nature of the hispidity on its lower leaf-surfaces. These have a conspicuously reticulate venation, often with 15-25 principal veins on each side of the midvein. While the areolae included by the reticulations of the lower leaf-surfaces are glabrate, the veins themselves are densely hispid with short, curved, sharp, closely investing setae.

Forbes 152-M, from woods near the type locality, differs from the other fruiting specimens cited in having capsules barely 1 cm. long and pedicels mostly about 1.5 cm. long. Enough evidence to warrant varietal segregation, however, seems lacking.

Labordia olympiana sp. nov.; *Labordia membranacea* sensu Rock, Indig. Trees Haw. Isls. 405, pl. 164. 1913 (non Mann).—Arbor parva, 3-5.5 m. alta. Folia iis *L. membranacea* similis sed setis brunneis forsit minus hispidae, basi magis rotundata, apice minus attenuata. Inflorescentia pauciflora, floribus iis

L. membranacea similibus, calycebus 8-11 longis. Capsula dimera, diverse conico-ovoidea vel ovoideofusiformis vel cylindrico-ovoidea, 2-4 cm. longa et 1.1-1.8 cm. crassa, valvis non earinatis apice non vel obsolete rostratis.

Specimens examined: *Joseph F. Rock*, Mt. Olympus Trail, Isl. Oahu, 1912 (Bish., 2 type sheets).

Rock (loc. cit.) included this form with *Labordia membranacea* Mann. His "three-flowered cyme," "valves not ridged at the back," and "Mt. Olympus trail, where it is a small tree 10 to 18 feet in height" are clearly taken from the data pertaining to the two type specimens of *L. olympiana*. His description of the calyx-lobes as being 1.5 cm. long is doubtless a misprint. The entire calyx of *L. olympiana* is under 11 mm. long (and in *L. membranacea* it is only about 6 mm. long).

Hillebrand (Fl. Haw. Isls. 292. 1888) seems to have been unfamiliar with this species. His description of the capsule of *L. membranacea* would appear *per se* to have been drawn really from *L. olympiana*'s capsule. On this point, however, his herbarium specimens of *L. membranacea* (Berl.) are conclusive. Only his Palolo specimen has any fruit at all and this a single rudiment of a year previous to that of collecting. The inflorescence had produced 3 capsules. Of these the reticulated fibrovascular system of the mostly disintegrated exocarp remains, capped in each case by a rudiment of the manifest capsular rostrum. In one instance the membranous interior shell of the capsule persists and is evidently the basis for Hillebrand's misleading description of the capsule for *L. membranacea*: "conico-elongate, about 12 lines in height, the pergameneous valves not ridged at the back."

More material of *L. olympiana* is much to be desired, that the foliar and floral characters, as distinguished from those of *L. membranacea*, may be more accurately drawn.

Labordia muiensis sp. nov.—Frutex pumilus \pm 3 dm. altus, ramis interdum prostratis radicanibusque aliter suberectis, ramulis angulatis glabrisque, stipulis sub 2 mm. altis saepe imperfecte connatis. Folia glabra inferne ad basin petiolatam sensim angustata, petiolo marginato 3-10 mm. longo; lamina elliptico-oblancoolata, pallida, apice subobtusae vel subacutae vel raro subacuminata, submembranacea, in herbario plerumque revoluta, 3-6.5 cm. longa et 8-15 mm. lata, nervis lateralibus principalibus utroque latere 4-6, supra subobsecuris infra moderate manifestis. Inflorescentia terminalis, non exserta, cynoso-corymboidea, plerumque 5-15-flora, saepius 2-3.5 cm. longa et 3-4 cm. lata, glabra (vel interdum ramulis laxae setulosa), bracteolis linearibus et circ. 4 mm. longis. Calyx profunde (usque vel fere ad basin) sectus, nunc glaber nunc hispidulus; lobis oblongo-linearibus vel lanceolatis, apice obtusis vel acutis, 3-5-nerviis, nunc (pro typo) 4-6 nunc etiam 5-10 mm. longis. Corolla extus glabrata vel pubescens, intus albo-hispida; tubo 1-1.5 cm. longo et 1-4 vel etiam usque ad 6 mm. crasso; lobis anguste vel late lanceolatis,

4-6.5 mm. longis. Pistillodium circ. 13 mm. longum; ovario siccio nigro, conico, sparsissime setuloso, circ. 5 mm. longo; stylo filiformi, inferne hispido, circ. 4-5 mm. longo; stigmatibus anguste obovatis, papillatopubescentibus, 3-4 mm. longis. Pistillum immaturum (maturum ignotum) circ. 8 mm. longum, ovoideo-conicum, basin versus subglabratum alibi perspicue denesque erecto-hispidum, stylo non distincto. Antherae lineares, steriles circ. 2 fertiles circ. 2.2 mm. longae.

Specimens examined: *Otto Degener* 10264, in dense, dark rain-forest, Pipe-Line Trail, Olinda, East Maui, June 21, 1927 (Berl.; Deg.; Del.; Field; Gray; Kew; Mo.; Mun.; Mus. V.; Par.; U.S.); *Joseph F. Rock*, Nahiku Stream, East Maui, May, 1911 (type, Bish.; cotypes, Gray; Mus. V.).

Rock had regarded this as a new species but did not describe it.

Labordia decurrens sp. nov. — Frutex vel arbor parva; ramulis gracillimis, griseo-brunneis, subteretibus, glabris vel moderate papillatis; stipulis subobsoletis, tantum circ. 1 mm. altis. Folia magna, petiolo plano vel inferne conduplicato 1-2.5 cm. longo; lamina late subspathulato-lanceolata, 1-1.8 dm. longa et 2-5 cm. lata, infra medium sensim usque ad basin plus minusve decurrentem angustata, apice acuta vel subacuminata, maxime membranacea, glabra, vena mediana plana et circ. 1.5 mm. lata. Inflorescentia (vetusta) paniculato-cymosa, glabra, \pm 6 cm. alta et \pm 7 cm. lata, usque ad circa 15-fructuata, ramis primariis oppositis et divaricatis, pedicellis ultimis gracillimis plerumque 1-1.5 cm. longis. Flores ad anthesin non visi. Sepala denum patentia vel subreflexa lanceolato-triangularia, glabra vel ad margines setulosa, apice plus minusve acuta, circ. 1.5 mm. longa. Fructus globoso-obovoides, numerosi et 6-7 mm. alti vel pauci et \pm 9 mm. alti, siccis atri, bipartiti, apice minutissime rostrati, extus sericeoluto-rugulosi sed aliter glabri, valvis non carinatis.

Specimens examined: *Horace Mann & William T. Brigham* 610 *pro parte*, Isl. Oahu, May 4, 1864-May 18, 1865 (type, Mo.; cotypes, Corn.; Field).

This species was confused by Mann with *Labordia Tinifolia* A. Gray. Indeed, Mann not only labeled the specimens of the type collection *L. Tinifolia* but applied his collection number 610 also to two other specimens (Bish.; Gray), both of which are true *L. Tinifolia*. *L. decurrens* differs enough, however, in the characters noted to be distinguished very easily from that species. The type collection lacks flowers, but the fruiting calyces are similar to those of *L. Tinifolia*.

Labordia Helli sp. nov.; *Labordia Tinifolia* var. γ . Hillebr. Fl. Haw. Isls. 293. 1888 (as to description and first cited material, a specimen from Kauai and found to be *Knudsen* 199 in the Hillebrand herbarium at Berlin). — Frutex vel arbor magnitudine mediana, glabra, ramis plus minusve griseis, teretibus, vix divaricatis. Folia superne parce conferta, petiolata; petiolis tenuibus, 7-15 mm. longis; laminis membranaceis, elliptico-oblongis vel obovato-oblongis, pallidis,

apice breviter acuminatis, basi cuneate contractis, margine integris vel obsoletissime denticulatis, laevibus, 4.5-10.5 cm. longis et 1.7-4 cm. latis, nervis lateralibus principalibus subobsoletis gracillimis plerumque 5-7 utroque latere. Flores paniculato-cymose dispositi, \pm 25 pro unica inflorescentia; inflorescentiae ramis oppositis, gracilibus, glabris, divaricatis; pedunculo 0.5-2 cm. longo, pedicellis denum \pm 1 cm. longis. Calyx glaber, circa 2 mm. longus; lobis ovato-cordatis, apice acutis, margine perspicue stramineo-flavidis, basi plus minusve auriculatis, saltem 1 mm. longis. Corolla crassiusculo-cylindrica; tubo externe glabro, interne albo-piloso, 6-8 mm. longo et usque ad 2.5 vel ad guttur 3 mm. crasso, interdum superne angustato; lobis cordato-ovatis, patentibus, acutis, in siccio marginaliter stramineis, circ. 1-7 mm. longis; antheris paulum exsertis; pistillodii capite dimidio exserto. Flores femine ad anthesin ignoti. Capsula ovoidea, apice perspicue acerrime mucronata, circ. 12-13 mm. longa, bivalvata; valvis tergo longitudinaliter reticulataque venosis.

Specimens examined: *Amos A. Heller* 2579, on the ridge west of the Hanapepe River, Isl. Kauai, Jul. 17, 1895 (type, Field; cotypes, Berl.; Bish.; Corn.; Gray; Kew; Minn.; N.Y.; Par.); *Heller* (similarly) 2579, same locality, Aug. 22, 1895 (Mo., 1 sheet staminate and 1 sheet fruiting material; N.Y., staminate; U.S., staminate and fruiting specimens on same sheet); *Valdemar Knudsen* 199, Isl. Kauai (herb. Hillebr. in Berl.); *St. John, Hosaka, Hume, et alii* 10990, tree 20 feet tall, alt. 800 feet, Napali coast, Hanakapiai, Isl. Kauai, Jan. 2, 1931 (Berl.; Bish.; Field); *St. John, Fosberg, & V. Oliveira* 13810, slender shrub, 8 feet tall, "flowers white, pendulous, hairy inside," alt. 3600 feet, Kumuvela Ridge, Waimea, Isl. Kauai, December 28, 1933 (Berl.; Bish.; Field; Par.).

Easily distinguished from *L. Tinifolia* A. Gray, with which it heretofore has been confused, by the shape and yellowish margins of its calyx-lobes and by the acutely ovoid fruits, these being (under a lens) conspicuously and reticulately lengthwise-nerved. The existence of a variety (*macrocarpa*) with fruits comparatively gigantic but with the same peculiarities of shape and external venation lends additional support (if such be needed) to the segregation of this form as a distinct species.

Hillebrand (*loc. cit.*) described his *Labordia Tinifolia* var. γ . in the few words: "Capsules ovoid, acute, 6" long." He cited "Kauai! Maui! Kaanapali! Lanai! leaves small obtuse, almost rounded at the base; Hawaii!" for the distribution.⁴

The Lanai material, as also that from Hawaii and West Maui, has been separated by me as *L. Tinifolia* var. *lanaiensis*. The material from the first cited locality—i.e., Kauai—is still extant in Hillebrand's herbarium (Berl.). It was collected by Knudsen, no. 199, and matches Heller's specimens closely. Heller (Minn. Bot. Studs. 1: 877. 1897) stated that his

⁴ His Lanai material itself (Berl.) enables us to recast the ambiguous and misleading description of its leaves to read: leaves small, their base obtuse or almost rounded. They are not obtuse at the apex.

specimens were obtained "from a medium-sized tree" in July.

Labordia Helli *macrocarpa* var. nov.—Frutex vel arbuscula ≈ 4.5 m. alta; capsula acuta, oblonga vel lanceolata ovoides, saepius 1.5–2.4 cm. longo.

Specimens examined: *Charles N. Forbes* 378-K, Kaholuamanu, behind Waimea, Isl. Kauai, September, 1909 (Bish.; Field; Kew); *Forbes* 721-K, Haupū Range, left-hand side of Kipu Kai Gap, Isl. Kauai, Nov. 1, 1916 (Field; Mo.); *Forbes* 962-K, Waimea Drainage Basin, west side, Isl. Kauai, Jul. 3–Aug. 18, 1917 (Bish.; Field; Par.); *Forbes* 1133-K, same locality and date (Bish.); *Heller* 2868, on Kaholuamanu, above Waimea, Isl. Kauai, Oct. 1–8, 1895 (type, U.S.): cotypes, Field; Gray; Mo.; N.Y.); *William Hillebrand*, Hawaiian Isls. (U.S.); *Joseph F. Rock* 5887, 5890, and 5891, trail to Opaewela, Kaholuamanu, Isl. Kauai, September, 1909 (Bish.); *Rock* 5888, Kaholuamanu, Sept. 7, 1909 (Bish.; Gray; Mus. V.); *Rock* 5912, growing as a shrub, trail to Opaewela Stream near Kaholuamanu, Sept. 7, 1909 (Bish.); *Rock* 17103, Kaholuamanu, October, 1916 (Bish., 3 sheets).

Heller (Minn. Bot. Studs. 1: 876. 1897) doubtfully referred his no. 2868, which constitutes the type-basis of this variety, to *Labordia pallida* Mann, with which it has very little in common. His specimens were collected "from a small tree, about fifteen feet high, with slender trunk, and spreading top." His printed labels all give Oct. 1–8 as the date of collection, but his published discussion (*loc. cit.*) gives the date as Sept. 25.

Labordia hirtella sororia var. nov.—Folia numerosa, obovata vel interdum ovali-oblonga ovatae, apice subabrupte vel subsensim acuminata, infra sparsim setulosa vel nisi secundum venas glabrata, plerumque 2.5–5 cm. longa et 1.3–2.6 cm. lata. Pedicelli (saltem supra) et sepala glabrata. Corolla tubo gracilis crassave (1.2–3 mm.), 8–10 mm. longa. Antherae florum masculinorum ochroleucae, lineares, circ. 2.2 mm. longae. Pistillodium inferne patenti-pilosum setis subalbis; stylo filiformi demum sepala facile (4–4.5 mm.) superante; stigmatate lineari vel graciliter obovato, circ. 2.5 mm. longo.

Specimens examined: *Charles N. Forbes* 103-M, Iao Valley, West Maui, June, 1910 (Berl.; Bish.; Field; Gray; Kew; Mo.; Par.); *Joseph F. Rock* 8151, Hawaiian Isls. (Bish.); *Rock* 8152, alt. 4000 feet, Honokowai Gulch, West Maui, Aug. 24, 1910 (type, Bish.: cotypes, Berl.; Field; Gray; Kew; Mus. V.).

Named in allusion to its apparently sister-like relationship to var. *microphylla* Hillebr.

Labordia hirtella haleakalana var. nov.—Folia oblonga vel obovata, abrupte breviterque acuminata, lamina 5–9 cm. longa et 2.5–4.5 cm. lata, infra minute subpubescentia praecipue secundum venas; petiolo glabro vel glabrato, 5–14 mm. longo. Calyx ut pro varietate *microcalyci* Hillebr. sed pubescens. Corollae tubus gracilis, 12–14 mm. longus. Pistillodii stylus calycem multo (≈ 9 mm.) superans; stigmatate lineari-oblongo, ≈ 3.2 mm. longo.

Specimens examined: *Joseph F. Rock* 8626, "Ukulele Waikamoi Haleakala," East Maui, October, 1910 (type, Bish.: cotype, Gray).

Labordia hirtella hispidior var. nov.—Ramuli dense antrorso-hispidi. Foliorum petiolus saepius valde hispidus, ≈ 1 cm. longus; lamina ovato-oblonga vel parce oblongo-subobovata, apice acuminata, basi late cuneata, supra glabra, infra manifeste hispida setis siccis arcuatis stramineis, 6–8 cm. longa et 2–3.5 cm. lata. Inflorescentia laxa, ≈ 20 -flora. Calyx glaber vel glabratus, 7–8 mm. longus. Pistillodii stylus sepala vix paulumve superans; stigmatate graciliter cylindrico ≈ 4 mm. longo. Capsula ignota.

Specimens examined: *Charles N. Forbes* 43-M, Hanakaoo, West Maui, May, 1910 (type, Bish.).

Labordia hirtella laevis var. nov.—Ramuli plus minusve crispo-alati; foliis glabris vel demum glaberrimis.

Specimens examined: *Joseph F. Rock* 16012, Kamoku, Isl. Molokai, April, 1918 (type, Bish.).

Labordia Tinifolia leptantha var. nov.—Arbor parva vel frutex habitu *Labordiae Tinifoliae* ipsi similis; ramulis gracilibus, teretibus, griseis. Folia glaberrima petiolata petiolis tenuibus 0.5–1.5 cm. longis; lamina elliptico-oblonga vel vix elliptico-obovata, apice acuta vel acuminata, basi acuta vel cuneata vel raro subrotundata, margine integra vel obscure subdentielata, membranacea, 4–8.5 cm. longa et 1.7–3.2 cm. lata, nervis lateralibus principalibus utroque latere 5–8. Corpus interpetiolare stipulare ovato-triangulatum, superne pubescens, 1–1.5 mm. altum. Inflorescentia cymoso-paniculata, subsessilis (pro plantis masculinis) vel manifeste pedunculata (pro plantis femineis), pedicellis ultimis glabris, primum gracillimis, 7–15 mm. longis. Bracteolae subulatae 1–2 mm. longae. Flores masculini (feminei ignoti): calycis circ. 2 mm. longi lobis sub medio connatis, triangulato-subulatis, glabris vel margine minutissime hispidulo-ciliatis; corolla angusto-cylindrica, externe glabrata, interne erecto-setosa, tubo 8–9 mm. longa et sub 2 mm. crassa, lobis triangulato-lanceolatis patentibus vel apice reflexis circ. 3 mm. longa; antheris pro parte minima exsertis; pistillodio apicaliter pubescenti clavato-dilatato demum pro circ. 1 mm. exserto. Capsula globosa vel subobovato-globosa, demum nigra, 4–6 mm. longa; valvis 2, tergo rotundis, apice minute mucronatis.

Specimens examined: *Anonymous* (*Otto Degener* distrib. no. 10241), trail at Nuuanu Pali "hairpin turn," Isl. Oahu, Nov. 20, 1926 (Deg.; Field); *Otto Degener* 10255, exposed slope, northeast of Nuuanu Pali, Nov. 20, 1926 (Berl.; Deg.; Field; Gray); *Degener* 10260, forest, half mile north of Keahikua, West Maui, Jul. 21, 1927 (Berl.; Deg.; Field; Gray); *Charles N. Forbes* 1062-O, east side of Nuuanu Valley, Isl. Oahu, October, 1910 (Berl.; Bish.; Field; Gray; Kew); *Forbes* 2306-M, ridge, left-hand side, Olowahi Valley, southwestern West Maui, May 10, 1920 (Berl.; Bish.; Field); *Dr. William Hillebrand*, Waihupe, Isl. Oahu (Berl.; U.S.); *Joseph F. Rock* 14062, Kalae, Isl. Molokai, May 25, 1918 (Bish., 2

type sheets of staminate, abundantly flowering material); *communis*. Heinrich *Wawa sub num.* 2283 (legit William Hillebrand), Isl. Oahu (Mus. V.).

Labordia Tinifolia parvifolia var. *primum* nominat.; *Labordia Tinifolia* var. β . Hillebr. Haw. Isls. 293. 1888.—Folia minora, petiolis 4–10 mm. longis; laminis plerumque oblongo-ellipticis, utrinque acuminatis, 2.5–4 cm. longis et 1–1.6 cm. latis, nervis lateralibus principalibus 3–5 utroque latere. Cymae pauciflorae, pedunculis tenuibus \approx 2 cm. longis, pedicellis capilliformibus 2–2.5 cm. longis, capsulis subnigris vel griseis 6–8 mm. longis. Flores masculini ignoti.

Specimens examined: Dr. William Hillebrand, Ka-laupapa Pali, central northern coast of Molokai (type, Berl.).

Numerous specimens referred in herbaria to Hillebrand's var. β . by various workers on the Hawaiian flora are found to differ sharply from Hillebrand's type, mostly in shape and size of leaves, and in shape, size, and abundance of capsules. The type consists of three small fruiting specimens; flowers are entirely lacking.

Labordia Tinifolia waialuana var. *nov.*—Petiolus 1–1.8 cm. longus; lamina subrhomboidae elliptico-oblonga, basi cuneata vel subacuminata, apice sub-obtus brevissime vel obsolete mucronata, 6–8.5 cm. longa et 1.7–3.2 cm. lata, nervis lateralibus principalibus 5–7 utroque latere. Inflorescentia saltem 3–vel 9-flora, tenuiter pedunculata pedunculo 2–3 cm. longo. Flores masculini (feminei non visi) pedicellati pedicellis usque ad 1.5 cm. longis; corolla basim versus 2.5 mm. crassa, tubo 7–8 mm. longa, lobis \approx 3 mm. longa; antheris non vel paulum exsertis.

Specimens examined: Horace Mann & William T. Brigham 562, Waiailua Mountains, Isl. Oahu (type, Gray: cotypes, Conn.; Field; N.Y.).

The type material is represented in the New York Botanical Garden by additional but very fragmentary specimens of fruiting material, on the same sheet with staminate flowering material. The capsules are as in var. *leptantha*. The sheet at Cornell University has two sprays, each with two triflorous inflorescences. Mann had designated these specimens at Cornell as a variety of *L. Tinifolia* and chosen a varietal name alluding to the triflorous habit. In his printed Enumeration published later on (Proc. Amer. Acad. 7: 197. 1887), however, he listed the collection as *L. Tinifolia*.

Labordia Tinifolia lanaiensis var. *nov.*; *Labordia Tinifolia* var. γ . Hillebr. Fl. Haw. Isls. 293. 1888 (excluding the Kauai or first cited material).—Foliorum petioli 4–14 mm. longi; laminis oblongis vel late oblongo-ovalibus vel rarius obovatis, apice obtusis vel subcautis vel etiam subacuminatis, basi late cuneatis vel raro fere subrotundatis, plerumque 4–8 (interdum usque ad 9.5) cm. longis et 2–4 (interdum usque ad 5.2) cm. latis. Inflorescentia tenuissime pedunculata pedunculo usque ad 3.5 cm. longo, pauci (\approx 9–12)-flora, pedicellis gracilibus. Florum (tantum masculinorum visorum) corolla inferne non vel parce dilatata, circ. 7 mm. longa, lobis \approx 2 mm. longis, pistillodio

paulum exserto. Capsula anguste vel oblonge ovoidae, apice acriter mucronata, 1.3–1.7 cm. longa.

Specimens examined: Charles N. Forbes 79-L, mountains near Koele, Isl. Lanai, June 9, 1909 (Bish.; Field; Kew; Mo.); Forbes 267-L, east end of Lanai, June, 1913 (Bish.; Field; Gray); Forbes 281-L, mountains at east end of Lanai, June, 1913 (Berl.; Bish.; Field; Kew; Mo.); Dr. William Hillebrand, Kaanapali, West Maui (Berl.; labeled by Hillebrand for his var. γ); Hillebrand, Isl. Hawaii, 1868 (Berl.); Hillebrand, Isl. Lanai, July, 1870 (type, Berl.); George C. Munro 35, "Waipooa," Isl. Lanai, March 27, 1915 (Bish.); Munro (similarly) 35, Kahoai, Isl. Lanai, July 2, 1914 (Bish.); Munro 430, pali above "Waipooa," Isl. Lanai, Mar. 27, 1915 (Bish.; Field); Munro 432, head of Maunalei by Mahana, Isl. Lanai, Mar. 27, 1915 (Bish.; Field); Joseph F. Rock 8000, alt. 2000 feet, dry forehills, Mahana Valley, Isl. Lanai, Aug. 1, 1910 (Bish., 2 sheets; Field; Gray; Kew; Mus. V.); Rock 8099, damp forest, Mahana Valley, Aug. 6, 1910 (Bish.; Gray; Mus. V.); Rock 8169, West Maui, August, 1910 (Bish.).

The type has small leaves, the blade commonly 3–5 cm. long and up to 2.4 cm. wide. The larger measurements in the description, as also the characters of the capsule, are taken from other cited specimens which do not appear varietally different.

Labordia Tinifolia Euphorbioidea var. *nov.*—Folia minora et numerosiora, petiolis plerumque 4–7 mm. longis, laminis plus minusve obovatis 2–4 rarius usque ad 5.3 cm. longis et 1.2–1.7 rarius usque ad 2.5 cm. latis, apice saepius obtusis vel rotundatis. Inflorescentia pauciflora vel subnumerosa-flora, pedunculo \approx 5–10 mm. longo inter 2 ramos stante. Capsula ut pro specie ipsa.

Specimens examined: H. M. Curran 66, Isl. Maui, April, 1911 (U.S.); Abbé Urbain Faurie 536, Haleakala, East Maui, August, 1909 (Bish.); Jules Remy 361bis, Isl. Maui, 1851–1855 (Gray); Joseph F. Rock 8616, alt. 2500 feet, in lower forest, Makawao, East Maui, October, 1910 (type, Bish.: cotypes, Gray; Mus. V.).

The small, mostly obovate, often crowded leaves give specimens of this variety a striking resemblance to those of several varieties of *Euphorbia Celastroides* and *E. multifloris*. Rock (Indig. Trees Haw. Isls. 406. 1913) describes the trunks of the trees in the forests above Makawao as straight.

Labordia Tinifolia Forbesii var. *nov.*—Folia numerosa, pallida, petiolo gracili plerumque 1–2 cm. longo; lamina diverse lanceolata vel subrhombaeo-lanceolata vel elliptico-oblongeolata sed raro latiore, apice attenuatus acuminata, 5–8 cm. longa et 1–3 cm. lata. Capsula globoso-obovata, 5–8.5 mm. longa.

Specimens examined: Charles N. Forbes 553-Mo, on slopes of Olukui, Wailau, Isl. Molokai, September, 1912 (type, Bish.: cotypes, Berl.; Bish.; Field; Gray; Kew; Mo.).

Labordia Tinifolia tenuifolia Degener & Sheriff, var. *nov.*—Arbor erecta, gracilis. Folia pallida, petiolo gracili 0.5–1.5 cm. longo; lamina lanceolata vel ellip-

tico-oblancoolata vel oblongo-subobovata, apice vix vel moderate acuminata, plerumque 4-6.5 (rarius -9) cm. longa et 1.5-2.2 (rarius 3.3) cm. lata. Flores numerosi (saepius 15-30), subcorymbose in inflorescentia pendula dispositi. Corolla flava neque vero aurantiaca, tubo tenui circ. 7 mm. longo et basin versus 1.3-1.7 mm. crasso, lobis 3-3.5 mm. longis; pistillodii stylo nigro, glabrato.

Specimens examined: *Otto Degener*, Isl. Molokai, May 25, 1928 (Berl.; Deg.; Field; Gray; Kew; Mo.; Mus. V.; Par.; U.S.); *Degener* 10272, erect, slender tree, 12 feet tall, in forest, Kahuaavi Gulch, Isl. Molokai, May 12, 1928 (type, Field, 2 sheets of staminate material: cotypes, Berl.; Brit.; Del.; Gray; Kew; Mo.; Mus. V.; Par.; U.S.); *Degener* 10300, Isl. Molokai, June 19, 1928 (Field, 2 sheets).

Labordia Timifolia honolulensis var. nov.—Folia principalia laminis saepius 4-5.5 cm. longa, alia saepe numerosa parvaque laminis tantum 2-4 cm. longa, omnia anguste vel moderate oblanceolata apice saepius acuminata. Flores ignoti. Capsula atra, valvis (rostro incluso) 5-7 mm. altis.

Specimens examined: *Otto Degener* 10254, Pauoa Flats, Isl. Oahu, Nov. 22, 1925 (Deg.); *Charles N. Forbes* (with *Mrs. G. E. Kelly*) 2388-O, on Pacific Heights ridge, Isl. Oahu, Aug. 1, 1916 (type, Bish.: cotypes, Berl.; Field).

Labordia Hedyosmifolia Hosakana var. nov.—Frutex 6-12 dm. altus, ramis multinodosis, ramulis angulatis dense tomentoso-hispidis; stipulis \pm 4 mm. altis, connatis, extus inferne hispidis, superne glabratis hyalinisque. Folia prope ramulorum apices disposita, petiolo dense hispidio 3-10 mm. longo; lamina nunc ovata nunc obovata, supra glabra et impresso-nervata infra elevato-nervata et secundum nervos plus minusve hispida, apice submucronata vel acuta vel etiam rotundata, basi rotundata vel plus minusve cuneata, marginibus saepe irregulariter revoluta, plerumque 1.5-6 cm. longa et 1-3.4 cm. lata. Inflorescentia sessilis, umbellato-contracta, foliis inclusa, 7-20-flora. Sepala distincta, lineari-oblonga vel lineari-subulata, extus glabrata vel sparsim albo-setosa, margine subdiaphano saepe perspicue ciliata, longitudinaliter 3-7-nervia, 7-14 mm. longa. Corolla gracilis vel infra paulo ventricosa, extus glabra vel sparsim setosa, circ. 1.5-2.5 cm. longa, tubo circ. 0.8-1.5 cm. longo, lobis lanceolatis interne albo-setosis. Pedicellus sparsim setosus, \pm 6 mm. longus, bracteola tenuissime elongato-lineari 7-10 mm. longa. Capsula globoso-ovoides, glabra, 3-valvulata, ovario maturo glabro et circ. 1 cm. longo, stylo (rostro) glabro vel sparsim setuloso circ. 3-4 mm. longo; valvulis superne acri-carinatis. Flores masculini ignoti.

Specimens examined: *Degener, Park, Potter, & Bush* 10152a, on wind-swept summit, Kipapa Trail, Koolau Range, Isl. Oahu, June 2, 1935 (Berl.; Deg.; Field; Gray; Kew); *Degener, Takamoto, & Martinez* 10542, shrub 2 feet tall, at wind-swept summit, C.C.C. Trail, Alea, Isl. Oahu, Mar. 15, 1936 (Deg.; Field, 2 sheets); *Francis R. Fosberg* 9729, on exposed ridge, alt. 860 meters, Kipapa Gulch, Waipio, Isl. Oahu,

Aug. 6, 1933 (Field); *Edward Y. Hosaka* 679, shrub 4 feet tall, on denuded ridge, alt. 2500 feet, Kipapa Gulch, south ridge, Waipio, Isl. Oahu, Jul. 4, 1932 (type, Bish.: cotype, Field).

This variety is named for Mr. Hosaka, who collected not only the type but also numerous other specimens of *Labordia*, all of which he very generously placed at my disposal. Some of the stems examined are closely invested with fine moss, and the whole habit is that of a very scraggly, dwarfed, much branched shrub. The umbellate-contracted, numerous flowered inflorescence and tomentose-hispid branchlets give an appearance suggestive of *Labordia Fagraeoides* var. *sessilis*. The more often oval and usually shorter, irregularly revolute leaves of var. *Hosakana*, however, also their more conspicuous venation and the elongate, narrow, frequently ciliate, acute sepals separate it satisfactorily from that variety.

Labordia Hedyosmifolia Rockii var. nov.—Folia minora, breviter petiolata petiolo saepius 1-5 mm. longo; lamina elliptico-oblonga, plerumque 2-4.5 cm. longa et 1-1.5 cm. lata, utrinque glabra. Inflorescentia circ. 3-flora. Corolla \pm 1.5 cm. longa, calyce dimidio brevior.

Specimens examined: *Rock & Hashimoto*, Eke-Honokahau, West Maui, August, 1910 (type, Bish.).

A variety at once distinguished by its small, narrow, entirely glabrous leaves, as well as by its smaller flowers. A sheet of *Mann and Brigham's* specimens (no. 434, in Field Mus.) of *L. Hedyosmifolia* var. *centralis* has a flowering spray with small leaves only 2-3.5 cm. long and at first sight suggestive of this variety. Its leaves, however, are more oval and definitely somewhat hispid beneath as in the larger, more typical leaves of the second spray on the same sheet.

Labordia Hedyosmifolia robusta var. nov.—Internodia terminalia hispida. Folia perspicue angustaeque cuneato-obovata, apice subabrupte rotundata et subacuminata, infra moderate adpresso-hispida, lamina plerumque 4-7 cm. longa et 1.5-3 cm. lata. Inflorescentia \pm 15-flora, cum fructibus 5-9 cm. lata, ramulis hispidis. Sepala saepius anguste lineares, 3-5-nervia, saltem dorsaliter hispida, apice acuta 0.7-1.2 cm. longa. Capsula (rostro incluso) circ. 1.8 cm. longa (corpore circ. 1.4 cm. longo); valvis perspicue supra medium cristato-carinatis; pedicello hispidio et plerumque 1.5-1.8 cm. longo.

Specimens examined: *Joseph F. Rock* 10015, Nalehu (Naalehu) forests, Isl. Hawaii, January, 1912 (type, Bish., 2 sheets: cotype, Gray).

Labordia Hedyosmifolia var. *Grayana* (Hillebr.) comb. nov.; *Labordia Grayana* Hillebr. Fl. Haw. Isl. 290. 1888 (ex syn. *Labordia Fagraeoides sensu A. Gr.*).

Labordia Hedyosmifolia Skottsbergii var. nov.—Internodia terminalia hispida. Folia petiolata petiolo 1-2 cm. longo; lamina obovata vel cuneato-obovata, terminaliter subabrupte rotundata et fere subacuminata, infra secundum venas moderate alibi sparsim adpresso-hispida, 6-11 cm. longa et 3.5-5.2 cm. lata. Inflorescentia aperta, hispida, \pm 5-flora. Calyx 1-2

1.5 cm. altus, sepalis oblongo-lanceolatis 3-7-nerviis, glabris vel glabratiss. Corolla vix 2 cm. alta.

Specimens examined: *Carl Skottsberg* 590, in forest above Pahala, south slope of Mauna Loa, Isl. Hawaii, Sept. 19, 1922 (type, Bish.).

The type had been determined by Skottsberg as *Labordia Grayana* Hillebr. (cf. Meddel. Göteborgs Bot. Trädgård no. 157. 1936). It differs at once, however, in having leaf-blades hispid (not glabrous) below, and much larger (6-11 cm. long and 3.5-5.2 cm. wide, not 4-8 cm. long and 2-3.5 cm. wide), also branches of inflorescence and terminal internodes of branchlets hispid (not glabrous).

Labordia Hedyosmifolia kilaueana var. nov.—Varietati *Rochii* similis. Internodia terminalia glabra. Folia elliptico-oblonga vel rarius oblonge oblanceolata, apice subacuta, utrinque glabra, lamina 3-5.5 cm. longa et 1.3-2 cm. lata, petiolo 2-6 mm. longo. Inflorescentia (floribus ignotis) demum glabra, contracta, cum fructibus sub 4 cm. lata. Sepala lineari-lanceolata, 3-7-nerviis, apice acuta, dorso glabra, demum 7-13 mm. longa. Capsulae valvae cristis adjectis vix 1.5 cm. altae.

Specimens examined: *Francis R. Fosberg* 10102, shrub 1 meter tall, in wet forest, alt. 810 meters, between Makaopuhi and Napau Craters, Chain of Craters, Kilauea, Isl. Hawaii, Aug. 29, 1933 (type, Bish.: cotype, Field).

Labordia molokaiana var. *lophocarpa* (Hillebr.) comb. nov.; *Labordia lophocarpa* Hillebr. Fl. Haw. Isl. 289. 1888 (exclud. Lanai plants).

Labordia molokaiana Munroi var. nov.; *Labordia lophocarpa* Hillebr. Fl. Haw. Isl. 289. 1888 (only as to Lanai specimens).—Ramuli ultimi internodiis terminalibus dense aciculato-setulosi. Folia parva, plerumque angusto-obovata, apice breviter-acuminata, infra adpresso-setosa; petiolo lato angustove, sub 8 mm. longo; lamina 3.5-6 (raro usque ad 7.5) cm. longa et 1-4 cm. lata. Inflorescentia 1-3-flora; corolla extus (tubo saepe dense et perspicue) hispidula.

Specimens examined: *Charles N. Forbes* 27-L, mountains near Koele, Isl. Lanai, June, 1913 (Bish.); *Forbes* 206-L, same locality and date (Bish.; Field); *Forbes* 242-L, mountains, east end of Lanai, June, 1913 (Bish.); *Dr. William Hillebrand*, Isl. Lanai, July, 1870 (type, Berl.); *George C. Munro* 229, Lanaihale, Isl. Lanai, May 17, 1914 (Bish.); *Munro* 413, same locality and date (Bish.); *Munro* 449, ridge below Lanaihale, May 17, 1915 (Berl.; Bish.; Field).

Labordia molokaiana var. *phyllocalyx* (Hillebr.) comb. nov.; *Labordia lophocarpa* var. *phyllocalyx* Hillebr. Fl. Haw. Isl. 290. 1888.

Labordia molokaiana congesta Degener & Sheriff, var. nov.—Ramuli saepe tetragoni angulis etiam subulati. Folia numerosa confertaque, pallida, breviter petiolata petiolo 2-6 mm. longo; lamina oblongo-ovata vel interdum obovata, apice acuta sed parce acuminata, glabra, 2-6 cm. longa et 1-3 cm. lata. Sepala exteriora late ovata, basi plus minusve auriculato-cordata et late substipitata, dorso glabrata, saepius 1-1.5 cm. longa. Bracteolae spatulatae. Corolla

1.3-1.5 cm. longa, extus plus minusve pubescens intus albo-setosa, tubo \pm 7.5 mm. longo, lobis oblonge linearibus.

Specimens examined: *Otto Degener* 10278, head of Waihanau Stream, Isl. Molokai, May 23, 1928 (type, Field: cotypes, Berl.; Deg.; Gray); *Degener* 10279, near Lalanui, Isl. Molokai, May 18, 1928 (Berl.; Deg.; Field; Gray).

This and the var. *setosa* are distinguished from *L. molokaiana* proper and its other varieties by their numerous, small, pale leaves, these much crowded toward the ends of the subulately tetragonal branchlets. The two varieties are apparently too closely alike in foliage to be recognized with certainty unless calyces are present.

Labordia molokaiana setosa Degener & Sheriff, var. nov.—Varietati *congestae* valde similis sed sepalis obovatis basin versus subsensim angustatis (vix cordatis vel auriculatis) dorso (inferne densissime superne moderate sparsimve) antrorsum fusco-hispidis paulo majoribus differt.

Specimens examined: *Otto Degener* 10282, in tapestry forest near top, west side of Waikolu Valley, Isl. Molokai, Apr. 21, 1928 (type, Field: cotypes, Berl.; Brit.; Field, 2 sheets; Gray; Kew; Par.; U.S.).

Labordia molokaiana Bryanii var. nov.—Ramuli aegro- vel subtetragoni, glabri. Foliorum petioli 1-8 mm. longi; laminae oblanceolatae, apice manifeste acuminatae, ad basin sensim angustatae, supra glabrae, infra moderate adpresso-hispidae. Inflorescentia saepius 3-flora. Calyx subsessilis vel demum breviter pedicellatus; sepalis late ovatis, longitudinaliter 5-9-nerviis, dorso dense vel demum moderate vel etiam subsparsim hispidis, 1.5-2.5 cm. longis. Capsula cristis perspicuis adjectis circ. 1.8 cm. alta.

Specimens examined: *Francis R. Fosberg* 10965, bush, 2 meters tall, flowers orange-yellow, leaves pale beneath, on wet, brushy ridge, alt. 880 meters, main divide north of Palikea, Waianae Mts., Honouliuli, Isl. Oahu, June 30, 1935 (type, Bish.: cotype, Field); *Edward P. Hume* 42, near spring, on wet, steep slope, alt. 3800 feet, Kaala, Waianaeauka, Isl. Oahu, Nov. 31, 1930 (Bish.); *Harold St. John* 12214, shrub, 15 feet tall, in moist woods near summit, alt. 3050 feet, Puu Kaa, Honouliuli, Waianae Mts., Isl. Oahu, Nov. 6, 1932 (Berl.; Bish.; Field); *D. LeRoy Topping* (*Otto Degener* distrib. no.) 2859, Kalena, Isl. Oahu, Sept. 14, 1924 (Deg.; Field).

Named for Mr. Edwin H. Bryan, Jr., Curator of Collections at the Bernice Pauahi Bishop Museum, to whom I am indebted for the lending of the first three collections cited, as well as for much other material lent me from the same institution.

Labordia Fagraeoides Humei var. nov.—Frutex \pm 3 m. altus; ramulis angulatis, glabris, ad apices moderate vel conferte foliosis. Folia minora et angustiora; lamina (saepe subrhomboidale vel oblonge) oblanceolata, infra medium saepe angusto-cuneate usque ad basin attenuata, coriacea, glabra vel facie inferiore pallidior saepe sparsim adpresso-setulosa, plerumque 4-7 (rarius -9.5) cm. longa et 1-2.5 (rarius

-3) cm. lata; nervis lateralibus principalibus tantum circ. 7 vel 8 pro unico latere. Inflorescentia sessilis, umbellata, 2-5-flora. Pedicellorum bracteolae 2 vel saepe tantum 1, paulo breviores. Calycis lobi ovati vel cordato-ovati, 2-6 cm. lati, glabri, acuti vel mucronati. Corolla carnosae, intense flavae; lobis lanceolato-linearibus, patulis, intus (praecipue inferne) albescentibus, apice acutis, 7-12 mm. longis; tubo sepala non superante, nunc glabro nunc (rarius) perspicue retrorsumque albo-setoso. Capsula 3-mera, glabra, 1-1.2 (etiam 1.8-2.1) cm. longa, globoso-ovoidae (vel vere ovoidae aut obovatae); valvis apicem versus mediane carinatis, moderate rostratis.

Specimens examined: *Degener, Martinez, & Salucop* 11124, rain-forest, Pig-God Trail, Punaluu, Isl. Oahu, Mar. 20, 1937 (Berl.; Brit.; Del.; Field, 2 sheets; Gray; Kew; Par.; U.S.); *Degener, Kwan Park, & Y. Nitta* 10234, Pig-God Trail, in forest half-way to summit, Punaluu, June 22, 1932 (Berl.; Deg.; Field; Gray; Kew; Par.; partly typical and with mature fruits, partly teratological and with monstrous, ovate sepals 1-2 cm. long); *Degener, Park, & Nitta* 10235, summit rain-forest, Pig-God Trail, Punaluu, June 22, 1932 (Berl.; Deg.; Field); *Degener, Park, & Manuel Kwon* 10238, forest half-way to summit, Pig-God Trail, Punaluu, Jan. 17, 1932 (Berl.; Deg.; Field); *Charles N. Forbes* (with *Dean Lake*) 1980-O, Waimanu Ridge, Isl. Oahu, Oct. 27-30, 1914 (Bish.); *Forbes* (with *Mr. Labouchere*) 2288-O, Kalihi Pali, Isl. Oahu, Feb. 16, 1916 (type, Bish.; cotypes, Berl.; Field); *Forbes & C. L. Thompson*, Koolauloa Mts. between Punaluu and Kaipapau, Isl. Oahu, May 8-13, 1909 (Field); *Francis R. Fosberg* 10896, corolla fleshy and deep chrome-yellow, leaves coriaceous and very pallid beneath, their veins pellucid, on steep bushy slope, alt. 740 meters, Waikane-Schofield Trail, Koolau Mts., Kahana, Isl. Oahu, May 12, 1935 (Field); *F. R. & V. O. Fosberg* 13736, shrub 3 meters tall, leaves thick and pallid beneath, their veins pellucid, flowers fleshy and orange-yellow, in wet forest, alt. 650 meters, Castle Trail, Punaluu Valley, Koolau Mts., Apr. 25, 1937 (Bish.; Field); *F. R. Fosberg & Edward Y. Hosaka* 13979, shrub 0.4 meter tall, leaves pallid beneath, their main veins pellucid, corolla fleshy and orange-yellow, in small, open bog, alt. 825 meters, above Kaipapau Gulch, crest of Koolau Mts., May 31, 1937 (Field); *E. P. Hume* 79, steep slope near top, alt. 600 meters, Punaluu, Jan. 11, 1931 (Bish.; one of two corollas with tube conspicuously and retrorsely white-hispid outside); *Hume* 439, shrub, alt. 1800 feet, on side of wooded hill, Punaluu, Punaluu-Kalanui divide, Dec. 21, 1931 (Bish.); *Harold St. John* 10074, shrub, alt. 1800 feet, Punaluu, Nov. 30, 1929 (Berl.; Bish.; Field).

Easily distinguished from the species proper by its narrower, usually much smaller, basally more narrowly cuneate, apically more gradually narrowed leaves, these with fewer lateral nerves and the lower surface much less hispid or even glabrate. The sepals, too, are much broader proportionately and more ovate than in the species itself. The fruiting specimens

examined have capsules mostly 1-1.2 cm. long, but a few capsules are found much longer. One collection (*Degener et al.* 11124) has, in addition to numerous small, mature capsules, a very few large, smooth, un-matured ones ranging up to 2.1 cm. long, exclusive of the styler rudiment (this about 4 mm. long).

Labordia Fagraeoides var. *Humei* forma *paniculata* f. nov.—Inflorescentia plerumque 5-vel 7-flora, breviter pedunculata pedunculo usque ad 6 cm. longo, paniculata ramulis (vel pedicellis) plus minusve oppositis, ≈ 2 cm. alta et ≈ 3 cm. lata. Flores paulo minores. Pedicellorum bracteolae saepius tantum 1.

Specimens examined: *Charles N. Forbes* 1440-O, ridge, each Nuanu Valley, Isl. Oahu, Feb. 8, 1910 (type, Bish.; cotypes, Berl.; Field; Mo.).

The leaf-blades on the few specimens seen are mostly 2-5 cm. long. However, in the variety proper some material is found with leaf-blades averaging as small. Thus the paniculate, more numerous flowered, pedunculate inflorescence offers the only distinction. In view of the remarkably close resemblance in numerous other respects to specimens of the variety proper collected in the same vicinity, the distinction would seem best considered as connoting a *forma*.

Labordia Fagraeoides conferta var. nov.—Frutex ≈ 1.5 m. altus; ramulis glabris acriter (petiolis deurentibus) angulatis. Folia prope ramulorum apices conferta, pallida, raro subsessilia plerumque sessilia, anguste obovata vel oblonge oblanceolata, apice moderate acuminata, glaberrima; nervis lateralibus principalibus plerumque 6-8 utroque latere. Inflorescentia sessilis, 3-5-flora. Sepala ovata vel late oblonga, normaliter 2-4.5 mm. lata (saepe monstrosa et multo majora). Corolla circ. 10-12 mm. longa. Capsula 1.3 cm. longa.

Specimens examined: *Otto Degener, Kwan Kee Park, & Wallace Hirai* 10237, in rain-forest, Waikane-Schofield Trail, Isl. Oahu, Apr. 4, 1931 (Deg.; Field); *Francis R. Fosberg* 8714, bush 2 meters tall, in scrubby, wet forest, alt. 900 meters, summit of Koolau Mts., above Kipapa Gulch, Waipio, Isl. Oahu, Sept. 18, 1932 (Field); *F. R. Fosberg & Edward Yataro Hosaka* 13922, shrub 1.5 meters tall, leaves pallid beneath, their main veins pellucid, on wet, bushy, windswept ridge, alt. 830 meters, crest of Koolau Mts., between Waikane Valley and Waikakalua Gulch, Isl. Oahu, May 30 and 31, 1937 (type, Field: cotype, Bish.); *Hosaka* 946, shrub 4 feet tall, alt. 2000 feet, wet, denuded ridge, south ridge of Kipapa Gulch, Koolau Mts., Isl. Oahu, Mar. 19, 1933 (Bish.; Field; Par.); *Hosaka* 968, alt. 2200 feet, on wet, denuded ridge, Kipapa Gulch, Apr. 16, 1933 (Berl.; Bish.; Field); *M. Yamaguchi* 1256 *pro parte*, alt. 2700 feet, south ridge, Kipapa Gulch, Jul. 4, 1932 (Bish.; Field).

The pale, terminal, numerous leaves of the type offer an illusory resemblance to those of certain varieties (e.g., var. *moenomiana*) of *Euphorbia Celastroides* Boiss. The other cited material generally is more scraggly, has its fewer and somewhat smaller (1.5-4.5 cm. long) leaves more localized in almost

rosette-like terminal clusters, and has flowers in anthesis (or (*Fosberg* 8714) matured capsules, but in technical characters and from geographic and ecological considerations appears to be identical.

Labordia Fagraeoides jugorum var. nov.—Ramuli foliis decurrentibus angulati, glabri. Folia sessilia vel rarius petiolo usque ad 1 cm. longo petiolata, spatulato-oblancoolata, glabra vel infra remotissime setosa, inferne usque ad basin vel petiolum sensim cuneato-angustata, apice acuminata, principalia saepe 7–10 cm. longa et 2–3 cm. lata. Inflorescentia sessilis, contracta, 3–7-flora, glabra. Calyx circ. 7–10 mm. longus, glaber, sepalis saepius lanceolatis et apice acribus. Corolla circ. 2 cm. longa.

Specimens examined: *Charles N. Forbes* 2208-O, Wahiawa-Kahana Trail, Isl. Oahu, Aug. 17–20, 1915 (Bish.); *Forbes* 2552-O, Konaheunui ridges, Isl. Oahu, Mar. 17, 1919 (Bish.); *Forbes* (with *C. M. Cooke*), Koolauloa Mts., between Punaluu and Kaipapau, Isl. Oahu, May 3–8, 1909 (type, Bish.).

Labordia Fagraeoides var. *sessilis* (A. Gray) comb. nov.; *Labordia sessilis* A. Gray, Proc. Amer. Acad. 4: 323. 1860.—Until now, *Labordia sessilis* A. Gray has been more or less enigmatic. Horace Mann (Proc. Amer. Acad. 7: 196. 1867) reduced it to *L. Fagraeoides* Gaud. Hillebrand (Fl. Haw. Isls. 290. 1888) modified Gaudichaud's description of *L. Fagraeoides* to read, "generally glabrous, but sometimes the young shoots and inflorescence coarsely pubescent," and then likewise reduced *L. sessilis* to *L. Fagraeoides*. Rock (Indig. Trees Haw. Isls. 406. 1913) maintained *L. sessilis* as a valid species "certainly distinct from *L. Fagraeoides*," but Rock's text makes it evident that he lacked a clear notion of what the genuine *L. sessilis* really was. Thus his opinion is of little value to us here. Skottsberg (Meddel. Göteborgs Bot. Trädgård 10: 163. 1936) has recently made a critical study of the two original specimens studied by Gray and refrains from attempting a definite disposition of them. He writes of *L. sessilis* Gray: "Possibly it belongs in the vicinity of *L. Fagraeoides*."

Fortunately I have been able to examine much illustrative material collected in the past few years upon Oahu by Degener, Fosberg, Hosaka, Swezey, and others, and have been able to reach a satisfactory understanding of Gray's type specimens. These latter were of two kinds. One had moderately small, widely oblanceolate leaves and mature capsules 1.2–1.5 cm. long, capped by a slender stylar beak about 3 mm. long. The other was a sterile shoot with gigantic, oblanceolate leaves, these up to about 15.5 cm. long and to over 4 cm. wide. On both, but conspicuously so on the sterile one, the distal internodes and the under surfaces of the veins were densely brown-hispid. There seems no question that the sterile shoot (which together with the other type material and numerous connecting specimens lies before me) is merely a more or less abnormal, perhaps teratological representative of the smaller-leaved fruiting form. Various collectors have distributed the two forms under the same

number and doubtless in many instances had collected them from the same bush or tree. Invariably the sprays with elongate leaves are found to lack flowers and fruit, or at most to have abortive and mutually more or less diverse flowers. At times the flowers are especially abortive and numerous.

Compared with typical *L. Fagraeoides*, the smaller-leaved sprays are found to differ chiefly in having the distal few internodes of the branchlets densely hispid, the leaves perhaps narrower and more oblanceolate, and the sepals sharper-pointed and more slender. *L. sessilis* is seen at once to be of coordinate rank with vars. *Humei* and *conferta* under *L. Fagraeoides*, to a variety of which it must therefore be reduced.

Labordia Fagraeoides waianaeana var. nov.—Ramuli obtuse tetragoni, glabrati. Folia (interdum anguste) obovata, usque ad 15 cm. longa et 6.5 cm. lata, tenuia, supra glabra, infra sparsim adpresso-hispida, apice acuminata, petiolo saepe indistincto usque ad circ. 12 mm. longo. Calyx circ. 1.3–1.5 cm. longus, sepalis tenuibus dorso (saepem infra medium) dense subadpresso-hispidis, nunc linearibus nunc late oblongis. Corolla \approx 2.2 cm. longa; lobis anguste lanceolatis, acerrime apiculatis, 10–12 mm. longis.

Specimens examined: *Francis R. Fosberg* 10367a, bush 1.5 meters tall, leaves white beneath, in wet, boggy forest, alt. 1225 meters, Puu Kaala, Waianae Mts., Waianaeauka, Isl. Oahu, Nov. 5, 1933 (Field); *Fosberg* 10369, leaves white beneath, flowers orange-yellow, bush 1 meter tall, alt. 1235 meters, same locality and date (type, Bish.: cotype, Field); *J. Arthur Harris* C-242120, shrub with yellow flowers, alt. 4000 feet, summit of Puu Kaala, Aug. 24, 1924 (Minn.).

The leaves of Fosberg's specimens are noticeably thin and bluish-green, doubtless from the method of drying employed. Harris C-242120 has the leaves mostly narrow-obovate. It has been determined by Skottsberg as *Labordia Fagraeoides* Gaud. (cf. Meddel. Göteborgs Bot. Trädgård 10: 154. 1936).

Labordia Fagraeoides Saint-Johniana var. nov. Habitu varietati *waianaeanae* similis. Inflorescentia plerumque 3–8-flora, hispida, pedicellis brevibus. Sepala late ovata vel rotundo-cordata, apice subobtusula vel acuta, dorso erecte adpresso-hispida, plerumque sub 1 cm. longa. Corolla circ. 1.5 cm. longa, lobis flavis reflexis lanceolatis ovatis apice acribus intus plus minusve albo-hispidis.

Specimens examined: *Francis R. Fosberg* 13013, shrub, 2 meters tall, flowers yellow, on moist, wooded ridge, alt. 800 meters, east ridge of Puu Kalena, Waianae Mts., Waianaeauka, Isl. Oahu, Mar. 22, 1936 (Bish.; Field); *Alfred Meebold* (Otto Degener distrib. no.) 10239, alt. 4000 feet, Waialua side of Puu Kaala, Waianae Mts., June 18, 1932 (Bish.; Deg.); *Harold St. John* 17605, petals reflexed and yellow, shrub, 5 meters tall, on wooded ridge, alt. 2800 feet, Puu Kawiwi-Puu Kaala Ridge, Waianae Mts., Mar. 31, 1935 (type, Bish.: cotype, Field).

Labordia Fagraeoides longispala var. nov.—Ramuli graciles, internodiis 1 vel 2 terminalibus moderate

hispidis. Folia oblongo-oblancoolata, apice facile acuminata, inferne in petiolum gracilem plus minusve setosum 5-11 mm. longum cuneate angustata, supra glabra, infra sparsim adpresso-hispida, lamina 5-8.5 cm. longa et 1.5-3 cm. lata. Sepala lanceolata vel late oblonga, plurinervia, glabrata vel irregulariter sparsimque adpresso-setosa, 1.4-1.8 cm. longa, corollam aequantia. Capsula ignota.

Specimens examined: *Charles N. Forbes*, on Kaaawa Valley ridges, Isl. Oahu, May 30, 1909 (type, Bish.).

A plant of peculiar aspect and deserving of more study. The type sheet bears three sprays, with a total of 4 inflorescences, these 1-3-flowered. The flowers are subsessile and subtended by apparently two distinct types of bracts: one kind linear and about 3-4 mm. long, the other kind very delicately filiform and often 1-2 cm. long.

Labordia Pagraeoides septentrionalis var. nov.—A varietate typica foliis paulo angustioribus infraque omnino glabris sepalis latioribus \approx 1.5 cm. longis differt. Corolla circ. 2.5 cm. alta, lobis lanceolatis acriter apiculatis. Capsula ignota.

Specimens examined: *Anonymous*, mountains of Punaluu, Isl. Oahu, Aug. 18, 1908 (type, Bish.); *Degener, Park, Topping, & Bush* 10236, shrub 4 feet tall, in rain-forest at summit, Pupukea-Kahuku, Isl. Oahu, May 17, 1931 (Field).

Labordia glabra orientalis var. nov.—Capsulae elongatiorae, ovoideae, 17-19 mm. longae (rostrum 2-3.5 mm. longo inclusio), valvis nunc 3 nunc tantum 2.

Specimens examined: *Charles N. Forbes* 1644-M, on ridge, left side of Kipahulu Valley, southeastern East Maui, Nov. 15, 1919 (Bish.); *Joseph F. Rock* 10344, Honomanu, northeastern East Maui, May, 1911 (type, Bish., 2 sheets: cotype, Mus. V.).

The Forbes material had been determined at Bishop Museum, apparently by Forbes himself, as a variety of *Labordia glabra* Hillebr., and the 2-valved capsule had been noted on the sheet ("capsule not 3-valved") Rock had made a special study of his own no. 10344 and determined it, under date of April 6, 1914, as *L. glabra* Hillebr. The elongate fruits are quite distinct, however, from the short, globose ones of the species proper (both type sheets of which are before me). It is evident that the Rock and the Forbes specimens represent a geographic variety native to East Maui and easily recognized, at least when fruits are present, from the species proper of West Maui.

Labordia membranacea exigua var. nov.—Folia eis speciei ipsius minora; petiolo 0.5-3 cm. longo; lamina oblonge vel elliptice oblanceolata, apice acuminata, infra medium usque ad petiolum plus minusve sensim angustata, 4-11 cm. longa et 1.6-3.7 cm. lata. Inflorescentia 1-7-flora. Calyx 7-8 mm. longus. Capsula ignota.

Specimens examined: *Joseph F. Rock*, Waikamoi ditch trail, Ukulele, East Maui, September, 1910 (Bish.); *Rock*, Honomanu, East Maui, May, 1911 (type, Bish.).

Easily distinguished from the species proper, of Oahu, by the smaller and proportionately narrower leaves.

Labordia kaale Fosbergii var. nov.—Frutex 2-3 m. altus, ramulis folisque pallido-viridis et glaberrimus. Petioli 1-2.2 cm. longi; laminis 8-10 cm. longis et 4-5 cm. latis. Inflorescentia paniculata laxaque usque ad 12 cm. lata, \approx 22-flora, pedunculo gracili 4-6 cm. longo. Capsula submatura ovoidea circ. 1 cm. alta.

Specimens examined: *Francis R. Fosberg* 13002 *pro parte*, shrub 2-3 meters tall, fruits greenish, on moist, wooded ridge, alt. 860 meters, east ridge of Puu Kalena, Waianae Mts., Waianaeuka, Isl. Oahu, Mar. 22, 1936 (type, Bish.).

Fosberg distributed also a mature-fruited specimen of *Labordia Tinifolia* A. Gray under this collection number 13002.

The variety is named in honor of Mr. Fosberg, who has already done much in the botanical exploration of the Hawaiian Islands and who has most generously placed his large collection of *Labordia* material in my hands for study.

Labordia kaale brachypoda var. nov.—Petioli 3-7 mm. longi; laminis 5-8 cm. longis et 2-4.8 cm. latis, utrinque glabris, nervis lateralibus principalibus utroque latere circ. 7. Inflorescentia 3-5-flora. Sepala glabra, 1-2 mm. longa, margine non diaphana. Capsulae submaturae conico-ovoideae, apice subrostratae, siccæ atræ, glabrae vel glabratae, circ. 1.2 cm. longae.

Specimens examined: *Charles N. Forbes* 1587-O, Popouwele, Waianae Mts., Isl. Oahu, Apr. 27, 1910 (type, Bish., 2 sheets: cotype, Mo.).

The type and cotype material had been variously suspected by Forbes of being his *Labordia kaale*, of representing a variety of *L. triflora* Hillebr., or of being typical *L. triflora* Hillebr. and (in Forbes' judgment) perhaps meriting, as such, a varietal status under *L. Tinifolia* A. Gray. The frequently cordate leaf-bases, the simpler inflorescence with its 3-5 instead of 10-15 or more flowers, the larger and more conic-pointed capsules, etc. all separate var. *brachypoda* from *L. Tinifolia*. The frequently contracted bases and the broader and more or less obtuse (not acuminate) apices of the more rigid leaf blades, the longer petioles (commonly 3-7 mm. not only 1-2 mm. long or almost lacking), the much shorter (1-2.7 cm. not 3-5 cm. long) and thicker peduncles, etc. distinguish var. *brachypoda* easily from *L. triflora*. The shorter leaf petioles (3-7 mm. not 0.8-2.1 cm. long), smaller leaf blades (5-8 cm. not commonly 8-13 cm. long, 2-4.8 cm. not 4-7 cm. wide) with their glabrous not finely tomentose lower surfaces and their usually 7 not commonly 8 to 10 lateral nerves to each side, the few flowers (3-5 not 9-25 in a cyme), their conic-ovoid, apically narrowed and rostrate, 10-12 (or more) mm. long (not obovate-globose, apically short-mucronate, 8-9 mm. long) capsules, and doubtless other characters will separate var. *brachypoda* from *L. kaale* proper.

Labordia kaalae mendax var. nov.—Glabrata vel glabra, laminis 6-8 cm. longis et 3.5-4.3 cm. latis, petiolis crassiusculis circ. 1.5 cm. longis. Sepala dorso glabra, marginibus inferne diaphana. Capsulae obovoides 8-9 (etiam 10) mm. altae.

Specimens examined: *Alfred Meebold*, alt. 3800 feet, Makaleha ridge, Kaala, Waianae Mts., Isl. Oahu, June, 1932 (type, Bish.).

In its leaves and fruits this variety somewhat deceptively suggests *Labordia Tinifolia* A. Gray.

Megalodonta Beckii Hendersonii var. nov. — A specie ipsa foliis omnino compositis (superioribus nunquam simplicibus lanceolatisque) differt.

Specimens examined: *Louis F. Henderson*, in 2-3 feet of water, Siltcoos Lake, western Lane County, Oregon, August 14 and 21, 1931 (type, Calif.).

This variety had been observed for some years by Mr. Henderson, who remarked that it uniformly lacked the undivided, lanceolate upper leaves that characterize *M. Beckii* proper. I am indebted to Dr. Herbert L. Mason, of the University of California, for opportunity to study the type.

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RELATIVE HUMIDITY VARIATIONS AFFECTING TRANSPIRATION¹

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MANY WORKERS have measured the influence of relative humidity on transpiration and have verified the generally accepted statement that water loss from plants is inversely related to the relative humidity. Their methods varied from growing plants in separate greenhouses in which the humidity was partially controlled to noting the effect on water loss from plants outdoors as the humidity fluctuated from day to day. These plants usually had their roots sealed in pots, and the water loss was determined by weighing the plants and pots. Several important factors were usually playing their rôles in influencing water loss, such as changes of temperature, sunlight affecting the temperature difference between the plant and the surrounding air, the movement of the air, the degree of saturation of the plant cells, the opening and closing of the stomata, the absorption or availability of water from the soil, and others, as well as the influence of the relative humidity. These experiments thus more or less indirectly indicated the effect of the relative humidity on transpiration. It should be possible to measure more directly the influence of relative humidity on water loss.²

Darwin (1914) applied grease to the lower epidermis of common cherry laurel leaves and then made incisions of known length in the upper epidermis. Transpiration rates were measured by a potometer, and the humidity was varied from 50 to 100 per cent by placing a bell jar over the laurel branches. With this apparatus he found that the curve for transpiration from these leaf incisions followed a linear line as the relative humidity was decreased. His experiments are subject to the criticism that they were carried out on wounded tissue. A method that would eliminate the influence of as many of the factors as

possible should be devised, and, unlike Darwin's experiments, uninjured plant tissues should be used.

A method was devised by which small portions of leaves of the same plant could be exposed to varying humidity conditions. "Humidity bottles" were made in the following way. The base was composed of a glass "preparation" dish having an outside diameter of 50 mm. and a height of 34 mm. (see C in fig. 1). Over the mouth of this dish was sealed an aluminum cap (A) by use of a rubber paraffin preparation (B). This cap was previously prepared by having the center raised and a hole of .95 cm. in diameter with an area of .71 sq. cm. punched through the raised portion. The area of the opening was approximately one twenty-fifth of the area of the inside of the "preparation" dish. The opening was stoppered with a tight-fitting cork stopper.

To use a "humidity bottle" sufficient sulfuric acid of known concentration was inserted by using a pipette to have the surface of the acid 2 cm. from the hole in the cover. The bottle was stoppered, weighed on an analytical balance, and taken to the plant to be used, and a support for it was arranged under the experimental leaf. The stopper was removed, the bottle placed upon the support, the lower epidermis of the leaf pressed against the edges of the opening in the bottle and the leaf fastened in place. Usually glass slides were used to weigh the leaf and hold it in contact with the edges of the opening. Care was exercised to avoid injuring the epidermis.

The "humidity bottle" was left in contact with the leaf for eight hours, after which the leaf was removed from over the opening and the stopper inserted. The bottle was then weighed. Variations in weight of the bottle were interpreted as water loss by the leaf to the bottle or water loss from the bottle to the leaf. The amount of water absorbed or lost during the interval of time the bottle was open to the air in starting and stopping an experiment was found to be negligible. Absorption of water by the sulfuric acid solution will decrease the concentration somewhat and so affect the relative humidity main-

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² Unless the temperature remains nearly constant, it is important to control or measure the water vapor saturation deficit of the air rather than the relative humidity of the air. See the Discussion in the present paper and the articles by Livingston (1917), Anderson (1936), Curtis (1936), and Leighy (1937).

tained. The change is not large, however, and thus not significant for an eight-hour period. One of these humidity bottles would hold approximately 50 grams of a 50 per cent solution of sulfuric acid. If in eight hours the solution absorbs .2 grams of water, which is a high figure for these experiments, the effect will be a change of less than .3 per cent in the relative humidity maintained by that solution.

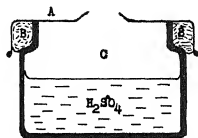


Fig. 1. Diagram of a "humidity bottle" used in these experiments. An aluminum cap (A) with a raised opening was sealed to a glass "preparation" dish (C) by a rubber paraffin sealing compound (B).

In these experiments an attempt was made to reduce all possible variables that may influence transpiration. Only healthy, active plants were used. Most of the plants were growing in pots, and care was exercised to have the plants well watered. Several fully matured leaves on a plant were selected for the experiments. A series of bottles was prepared and then placed in contact with these leaves. In order to reduce the influence of individual leaf variation, the bottles were rotated on these leaves on succeeding days. The rest of the plant, not directly subjected to the conditions of the experiments, was exposed to the humidity, temperature, wind, diffuse light, and other factors of the laboratory or greenhouse. The plant was shaded from direct sunlight by a white cloth screen.

The opening and closing of the stomata was the factor affecting transpiration least controlled. A tendency for the stomata to close was especially noticeable over the bottles having the high sulfuric acid concentrations. Stomata were examined and measured, and it was found that many of them were closed over the bottles containing 95 per cent sulfuric acid. In some leaves over lesser concentrations of sulfuric acid, the stomata would also close. In order to have a low relative humidity situation and yet not have this undesirable effect, other drying substances were tried. It was found that pellets of potassium hydroxide and sodium hydroxide could be used. Even with a low relative humidity within the bottle the stomata remained open. Apparently the closing of the stomata was not due to the low relative humidity within the bottles.

The following is a probable explanation of the difference in the behavior of the stomata over the sulfuric acid and the hydroxides. The opening and closing of the stomata is associated with changes in the acidity of the guard cells as explained by Sayre (1926) and Searth (1926). If, as was postulated,

TABLE 1. Water absorbed or lost by the substances in the "humidity bottles" from moist air or from the leaves of plants. The data given are averages for the indicated number of determinations.

Solutions or substances used to maintain relative humidities. Water absorption or loss by these solutions or substances given in grams per sq. cm. for an eight-hour period											
Plant leaves or materials used	Water: humidity value 100%	Sulfuric acid solutions						KOH pellets: humidity value 1%	NaOH pellets: humidity value 1%	Temperature	No. of determinations
		20%: humidity value 73%	35%: humidity value 42%	50%: humidity value 18%	65%: humidity value 3%	85%: humidity value 1%	95%: humidity value 1%				
Humid air	—001	.051	.120	.167	.194	.205	—	—	—	18-24°C.	2
Humid air001	.068	.153	.222	.255	.265	.280	.271	.271	20-31°C.	2
Purple coleus ..	—047	—011	.020	.053	.065	.057	.090	.076	.076	18-27°C.	15
Yellow coleus ..	—019	.011	.039	.059	.066	.058	.095	.085	.085	20-28°C.	9
Hibiscus	—051	—023	.019	.047	.059	.051	.077	.074	.074	20-28°C.	8
Cotton	—030	.002	.065	.105	.122	.104	—	—	—	18-27°C.	5
Lantana	—011	.027	.067	.083	.088	.068	.126	.120	.120	18-26°C.	6
Gladium	—018	.044	.085	.104	.091	.086	.164	.176	.176	24-31°C.	3

this acidity is due to differences in the concentration of the carbon dioxide in the guard cells, then the stomata over the hydroxides should tend to remain open. Guard cells have a low concentration of carbon dioxide when the stomata are open. As the hydroxides are effective in absorbing and reacting with carbon dioxide, the obtaining of it by the cells from the air in the "humidity bottles" with the hydroxides is improbable, and any accumulation within the guard cells will tend to be lost. As a result the stomata should tend to remain open.

The solubility of carbon dioxide in sulfuric acid is low, as shown by the *International Critical Tables* (1928). As no reaction occurs between them, the carbon dioxide in solution and in the air over the sulfuric acid should reach an equilibrium. The guard cells can obtain carbon dioxide from the air in such "humidity bottles," and they can also accumulate that released by cell activity. As a result of the increased carbon dioxide there is increased acidity, and the stomata close.

The early closing of some of the stomata over the sulfuric acid solutions of high concentration, however, is not explained. It might be explained if there is some release of sulfur trioxide vapor from the sulfuric acid. This seems probable from the data given in the *International Critical Tables* (1928). Sulfur trioxide even in very low concentrations would have a definite acid effect on moist cell surfaces and should influence the behavior of the guard cells. Observations of the closed stomata of several sets of plant leaves floated on very dilute sulfuric acid solutions tend to verify this conclusion. This agrees with Sayre's (1926) observations.

For these experiments commercial 95 per cent sulfuric acid (specific gravity 1.84) was used as stock. Volume percentage solutions of 20, 35, 50, and 65 were made from the stock solution. These solutions and bottles containing water and 95 per cent sulfuric acid gave a relative humidity series of 100, 73, 42, 18, 3, and near 1 per cent as worked out by Wilson (1921). These six "humidity bottles" and two others, one containing potassium hydroxide pellets and the second sodium hydroxide pellets, made a series of eight that constituted the experimental series. These bottles were usually prepared before 8 a.m. and then were placed in contact with the leaves of the plant between 8 and 8:30 a.m. The experiment was usually run for an eight-hour period.

For comparison of the results obtained with plants, these same bottles were set up in large moist chambers and the amount of water absorbed by the bottles from the humid air noted. The data obtained from these moist chamber experiments and from the experimental plants are presented in table 1. All data are calculated and presented as averages for an eight-hour period for one square centimeter of surface. These data are also presented in several graphs. The first graph (fig. 2) gives the results for the moist chamber experiments. The lower or less steep curve was obtained on Dec. 11-13, 1936, with the laboratory temperature fluctuating between 18° and 24°C. Each

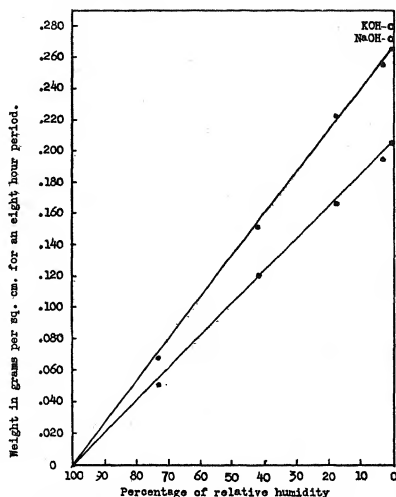


Fig. 2. Water absorption by known sulfuric acid solutions and hydroxide pellets from humid air.

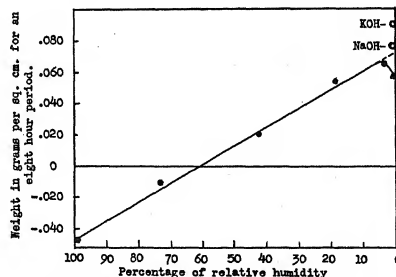


Fig. 3. Water absorption or loss for purple cactus. 15 determinations per point.

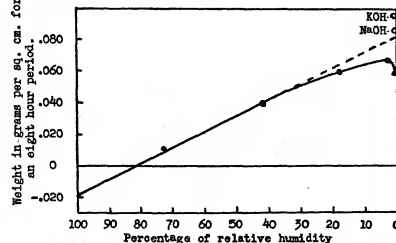


Fig. 4. Water absorption or loss for yellow cactus. The average of 9 determinations given at each point.

point on the curve is the average of two determinations for a 44-hour period. The second or steeper curve was obtained on August 12-14, 1937, with a temperature fluctuation of 26° to 31°C. Each point on this curve is the average of two determinations for a 46-hour period. The results for the two hydroxides used in these experiments ran higher than for the sulfuric acid. Other experiments corroborated these results. This difference is no doubt due to the increased absorption surface of the pellets of hydroxide over that of the sulfuric acid solutions. In all these experiments it is seen that there is an inverse linear relationship between the water absorbed by the bottles from moist air and the relative humidity maintained.

The plants used in these experiments were purple coleus and yellow coleus, both forms of *Coleus Blumei*; *Hibiscus Rosa-sinensis*; cotton, *Gossypium hirsutum*; *Lantana Camara*; and *Solanum Dulcamara*.

The first graph (fig. 3) for plant results is that for purple coleus. Each point on the curve is the average of fifteen determinations of eight hours each. The experiments were all made on plants in a south window in the laboratory. The environmental variations were those of a class room which was occupied by classes most of the day and during the time of an experimental run. The temperature in the window fluctuated from 18° to 27°C. but most of the time it was at or near 22°C. The relative humidity in the room as determined by the psychrometer method was usually near 30 per cent. Fluctuations from 22 and up to 50 per cent were observed. The light conditions were those for the months of December, January, and March. On days of sunshine a cheesecloth screen shaded the plant from direct sunlight.

In these experiments the major portion of the leaf was exposed to the conditions of the room. Only a small part of the leaf was actually exposed to the known relative humidity maintained by the bottle. The results from the several sets of experiments were similar. Those plant leaf portions exposed to the bottles maintaining high relative humidities did not lose water but actually absorbed water vapor. The bottles maintaining low relative humidities gained in weight, indicating that they absorbed water vapor from the leaves. These data show there is an inverse linear relationship between water loss and the relative humidity. By using the data obtained from the bottles of sodium hydroxide and potassium hydroxide, it can be seen that the water loss rate should continue in a straight line as indicated by the dotted extension to the graph. The closing of the stomata over the bottles having the higher concentration of sulfuric acid—i.e., maintaining the lower relative humidities—apparently does affect the rate of water loss from those leaves.

The results for yellow coleus (fig. 4) were obtained by experimenting on the plants in the greenhouse. Attempts were made to pick similar days for the experiments. Days of sunshine were selected, and the plants were shaded from the direct sunlight with a

cloth screen. The greenhouse temperature was usually near 22°C. and the relative humidity near 55 per cent; these conditions, however, fluctuated somewhat. The results, though more regular, show a curve similar to purple coleus. Each point on the curve is the average of 9 determinations of eight hours each. These results also show the decreasing rate of water loss from leaves over the stronger sulfuric acid solutions. Portions of some of the leaves examined showed that many of the stomata were closed, and the number was proportional to the concentration of the sulfuric acid within the bottle. A dotted extension on this curve is shown because of the position of the hydroxide points.

The curves for *Hibiscus* and cotton (fig. 5) were obtained by experimenting on plants in the greenhouse. The environmental conditions for these plants were similar to those for yellow coleus. Each point on the curve for *Hibiscus* is the average of 8 determinations. This plant in particular shows a curve similar to that of coleus, but the whole curve is shifted toward the right. Apparently the air in the intercellular spaces of the *Hibiscus* leaf is less humid than that for coleus, for the leaves absorbed more water in the region of higher relative humidities and lost less water to the bottles maintaining the lower relative humidities. Cotton was one of the first plants used in these experiments, and at that time the hydroxide bottles were not used. For this reason no dotted extension is shown on the curve. Each point on the curve is the average of 5 determinations.

The results for the *Lantana* curve (fig. 6) are the averages of 6 determinations and were obtained in conditions similar to those for purple coleus. The experiments were all performed in the same south window of the laboratory.

The last curve shown (fig. 7) is that for *Solanum Dulcamara*, and the data were obtained by experimenting upon plants growing outdoors in the shade on the north side of the main building. They received direct sunlight only in the early morning and late afternoon. The leaves used were on a vine within a few inches of the ground. The temperature there ranged between 24° and 31°C. and the relative humidity near 60 per cent. Each point on the curve is the average of three eight-hour determinations.

DISCUSSION.—The method used in these experiments is a static diffusion method. For this reason one cannot say that a leaf over a "humidity bottle" having a known sulfuric acid solution is exposed to a specific relative humidity. If a leaf loses some water into the bottle, immediately the relative humidity is changed. One can say that at a distance of two centimeters from the leaf is a solution capable of maintaining a definite relative humidity. Even here diffusion plays a rôle, for if a solution absorbs water at its surface, time is required for the solution to come to an equilibrium. The surface that has absorbed water molecules will maintain a higher relative humidity than is true for the whole solution. Even with these considerations the "humidity bot-

ties" maintain a relative humidity series as demonstrated by the absorption of water vapor from humid air. The pellets of hydroxide, having more surface than the sulfuric acid solutions, will absorb water molecules the faster. In comparing the absorption amounts from moist air and from leaves, it is seen that the vapor loss by the leaves does not tax the limits of absorption of the bottles.

Except for the dependence of these experiments upon diffusion into still air for the escape of water vapor from the leaf, they favor a fairly accurate measurement of water loss. As only a small fraction of a leaf was exposed to the conditions of the experiment, the limits of water absorption or of water transfer through the plant were never approached. Most of the plant was exposed to the usual laboratory or greenhouse environment, and the cells of the leaves should have been in nearly normal conditions. The experiments could be performed on uninjured plants, which is in contrast to Darwin's method. Some leakage of water vapor might take place between the rim of the opening and the leaf. The amount would be small or negligible, however, as most of the leaves showed a slight impression on the lower epidermis as made by contact with the rim of the bottle. These contacts seemed quite vapor-proof. Only the rim of the "humidity bottle" and glass slides usually touched the leaves. By using glass slides to hold the leaf in place, the light condition of the experimental leaf was not much different from that for the rest of the plant. Direct sunlight was avoided in these experiments to prevent the heating effect of such light. As these experiments were run for an eight-hour period, the results obtained represent the total for that time. The results represent the water loss from leaves through the middle of the day or period of greatest water loss. This method cannot be used for brief determinations, as the weight differences become too slight. For measurements over longer periods of time the method is satisfactory.

These experiments show that the transpiration rate from the leaves studied is an inverse, linear function of the relative humidity and may be expressed as a constant for each plant. For every 10 per cent decrease in relative humidity, the results for purple coleus show an increase of 11.7 units of water loss. By the same method yellow coleus has the constant 10.1, *Hibiscus* 11.8, *Lantana* 13.2, cotton 16.2, and *Solanum* 17.6. The steepness of the curves—i.e., the rate of water loss from the several plants—differ as shown by these constants. The two kinds of coleus and *Hibiscus* have similar curves and show a moderate rate of increase with changes of relative humidity. These plants are frequently used as greenhouse plants. Cotton and *Solanum* show a greater change in water loss due to changes of relative humidity. These plants are outdoor plants.

The data presented in these experiments may be interpreted in terms of water vapor saturation deficit or vapor pressure gradients, as suggested by Livingston (1917), Anderson (1936), Curtis (1936), and

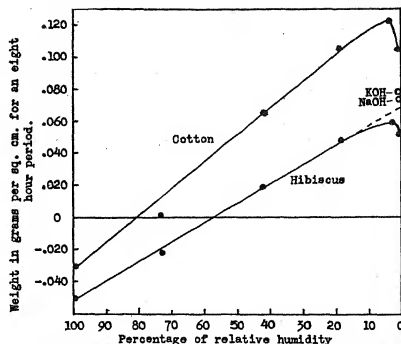


Fig. 5. Water absorption or loss for Hibiscus and cotton. 8 and 5 determinations per point respectively.

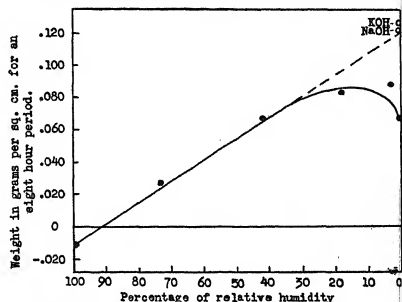


Fig. 6. Water absorption or loss for Lantana.

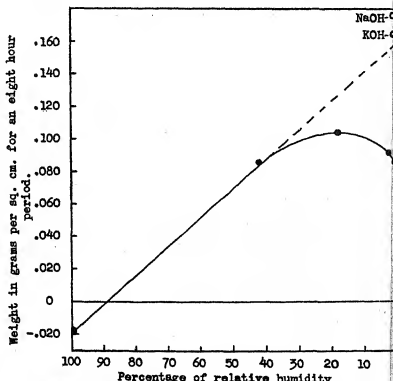


Fig. 7. Water absorption or loss for Solanum. 3 determinations per point.

Leighy (1937). If the temperature is considered a constant—for an attempt was made in these tests to hold the temperature of the laboratory or greenhouse at 22°C. during the eight-hour experiments—there is an inverse ratio between the relative humidity and the saturation deficit. For every mm. increase in saturation deficit or vapor pressure gradient purple coleus showed 5.9 units of water loss, yellow coleus 5.1, *Hibiscus* 5.8, *Lantana* 6.7, and cotton 8.2. If the temperature constant for the *Solanum* experiment was 27°C., then it showed 6.6 units of water loss per mm. saturation deficit. No attempt was made to interpret the temperature fluctuations during these experiments in terms of varying water loss. The influence of temperature becomes a complicated calculation where fluctuations occur even if the same relative humidity is maintained. The authors mentioned above have discussed this phase of the water loss problem.

Some of these results compare favorably with those obtained by Darwin (1914). His results are for one plant, common cherry laurel, and his experiments were performed with relative humidity variations ranging from 50 to 100 per cent. The results for cherry laurel show a constant of 10.7. Cherry laurel, the two kinds of coleus, and *Hibiscus* thus show very similar curves. The positions of the curves, however, are different. Darwin used cherry laurel twigs with leaves in a potometer, and he had the whole twig exposed to the known relative humidity condition. With this method he obtained water absorption by the twig which he interpreted as water loss from the leaves even if it were in an atmosphere of 100 per cent relative humidity. The plants in these experiments were for the most part in the environment of the laboratory or greenhouse. Only small portions of the leaves were exposed to 100 per cent relative humidity, and in all cases there was water absorption by the leaves.

The rates obtained for the bottles with low relative humidity compare favorably with those of Blaydes (1928). Blaydes used standardized cobalt chloride paper with which to measure the rate of water loss from various leaves. He obtained many rates ranging between 1 and 3 grams of water loss per 100 sq. cm. per hour. If the data for sodium hydroxide in these experiments are transposed into grams per 100 sq. cm. per hour, the results are: purple coleus .95 g., *Hibiscus* .925 g., *Lantana* 1.14 g., and *Solanum* 2.2 g. For *Solanum Dulcamara* he had a result of .61 g. for data collected in October, and these experiments show 2.2 g. for August.

In the region of high relative humidity, there was not a water loss but a water vapor absorption by the leaves in these experiments. As the plants were exposed to the laboratory or greenhouse conditions, transpiration caused a deficit in the water content of the leaf cells. The cells losing water by transpiration obtained water from the root and the roots from the soil. In this transfer of water there is a tension set up in the tissues due to the absorption lag, as

explained by Kramer (1938). If the tissues of the leaf are under tension due to a deficit of water, then it seems plausible that they should be able to absorb water. This they do, as is shown by the results of these experiments. This conclusion agrees with Wood (1925) who found that leaves absorbed water vapor quite freely from humid air. Williams (1932) and others have found that leaves absorb liquid water freely.

The line or curve that connects the points of water absorption in the region of high relative humidity with those of water loss by the leaf in the region of low relative humidity shows the influence of humidity on transpiration. The plant in the laboratory or greenhouse is subject to transpiration. This water loss causes a tension to exist in the tissues. The point on the curve where the line passes through the zero line of water loss from the leaf represents in terms of relative humidity the state of the water vapor in the intercellular spaces as caused by this tissue tension. It is at this point that the relative humidity maintained by the "humidity bottle" equals the tendency of the leaf to lose water. This point represents approximately the relative humidity of the stomatal openings and adjacent intercellular spaces, as most of the water is lost from leaves through the stomata during the daytime.

Purple coleus has 61 per cent and *Lantana* 91 per cent as the relative humidities of the intercellular spaces with the plants in a room having a relative humidity of 30 per cent and a temperature of 22°C. Yellow coleus has 82 per cent, *Hibiscus* 57 per cent, and cotton 81 per cent as that point with the plants in a relative humidity near 55 per cent and a temperature near 22°C. *Solanum* has 89 per cent as that point with the plant in an outdoor environment of 60 per cent relative humidity and a temperature of 27°C. These figures for the relative humidity of the intercellular spaces of leaves are much lower than the one figure of 99.2 per cent suggested by Shaw (1933). He used the turgor deficit of cells as a method of measuring the relative humidity of the intercellular spaces.

If, as has been suggested by Curtis (1936), the cell wall or protoplasmic membrane has a low permeability to water, and loss of water vapor to the atmosphere is rapid, the relative humidity of the intercellular spaces may fall considerably below 100 per cent. In these experiments this may be the explanation for the low relative humidities of the intercellular spaces as contrasted with the cell turgor deficit method of Shaw. More data are necessary in the region of high relative humidity to determine accurately the condition of the vapor of the intercellular spaces of leaves. The method of these experiments seems plausible for such determinations.

SUMMARY

Small portions of mature leaves were exposed to various atmospheres of known relative humidity. These were maintained by fastening the leaves over

"humidity bottles" containing sulfuric acid solutions of different concentrations or pellets of sodium or potassium hydroxide. The remainder of the plant was exposed to the usual environment of the laboratory or greenhouse. The plants used were purple and yellow coleus, *Hibiscus*, cotton, *Lantana*, and *Solanum Dulcamara*.

The water loss from the leaves of these plants was an inverse, linear function of the relative humidity over the entire range from 100 per cent to nearly zero relative humidity. The rate of increase of water loss with changes of relative humidity may be expressed as a constant, and it differs for the several plants.

Purple coleus has 11.7, yellow coleus 10.1, *Hibiscus* 11.6, *Lantana* 13.2, cotton 16.2, and *Solanum* 17.6 increase in units of water loss for every 10 per cent decrease in relative humidity. As the relative humidity increased, the transpiration decreased until at high humidities the leaves absorbed water instead of losing it. The point where transpiration ceases due to the high relative humidity is suggested as the relative humidity of the intercellular spaces of the leaf.

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CHROMOSOME BEHAVIOR IN TRIPLOID DATURA. III. THE SEED^a

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THE PRESENT is the third and final paper in a series having to do with the behavior of chromosomes in *3n Datura*. The first³ dealt with the male gametophyte. Due to a number of causes, including abortion of pollen grains, failure of pollen to germinate, and abnormal growth of pollen tubes, a marked elimination of chromosomally unbalanced male gametophytes takes place with a corresponding increase of *1n* gametes. In consequence, about 90 per cent of the offspring of a male back cross ($2n \times 3n$) are *2n* individuals, which is more than 3,000 times the number to be expected on random assortment of the *3n* chromosomes.

The second paper,⁴ which dealt with the female gametophyte, showed that lagging chromosomes during meiosis bring about a decrease in the more highly unbalanced female gametophytes with a consequent marked increase in the *1n* gametes over the number expected on random assortment. The condition of the female gametophyte is of more significance for the surviving offspring than that of the male gameto-

phyte, since the female is able to transmit genetic disharmonies which would be fatal to the male. It is for this reason that the offspring from female back crosses of a *3n* parent have a higher proportion of individuals with extra chromosomes than the offspring from male backcrosses. The present paper deals with chromosomal behavior in the seed and gives evidence of additional factors to those discussed in the previous papers which are responsible for the high proportion of *2n* individuals in the offspring from *3n Datura*.

The differences between the male and female gametophytes in a typical *2n* plant are shown in figure 1. A diagram of a flower after pollination is shown in the upper center of the figure. The diagram is simplified by representing only a single chromosome in each haploid set. At the left and right are shown the divisions giving rise respectively to the male and female gametophytes and gametes, and at the lower center is shown the formation of zygote and endosperm. The chromosomes of the two sperm cells (male gametes) are unshaded. The chromosomes of

^a Received for publication June 13, 1938.

the egg cell (female gamete) are distinguished by crosslines. The chromosomes of the two polar nuclei are dotted. The zygote contains one female ($1n$) gamete and one male ($1n$) gamete and becomes therefore a $2n$ cell. The endosperm is formed from the fusion of the two ($1n$) polar nuclei from the female with a ($1n$) sperm cell and is therefore $3n$. If the diagram had represented the chromosomes in a $3n$ instead of in a $2n$ plant, the chromosome condition in zygote and endosperm would have been different, as will be shown shortly.

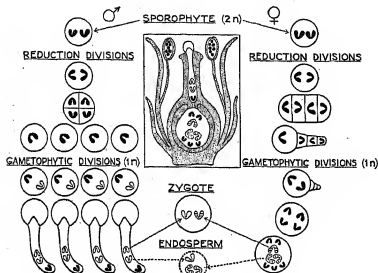


Fig. 1. Diagram of flower showing $2n$ and $1n$ generations.

FERTILIZATION.—Fertilization was studied in young capsules following the cross $3n \times 2n$. Six to eight days after pollination the capsules were fixed, sectioned, and stained, and other capsules from this cross were left as controls.

If an ovule becomes fertilized following a $3n \times 2n$ pollination, the number of chromosomes contributed by its female gamete can be calculated from counts either in the developing proembryo or in the endosperm. The latter is much better for chromosome counts because its cells are more numerous and much larger in size than those of the proembryo. If the 12 chromosomes brought in by the male gamete are subtracted from the number of chromosomes found in a cell of the endosperm, half of the remainder will represent the number of chromosomes brought in by the female gamete. In figure 2A, B, C, are shown three metaphase plates from the endosperms of three fertilized ovules. In figure 2A, where the number of chromosomes is 38, the female gamete had apparently $n + 1$ chromosomes [$(38 - 12) \div 2 = 13$]. That gametes with nuclei containing over $1n + 1 + 1 + 1$ chromosomes can become fertilized is seen in figures 2B and 2C. The number of chromosomes in the first is 44, in the second 54. It is evident that the female gamete in the first case must have had 16 chromosomes and in the second 21 chromosomes. Counts in endosperms of other ovules gave evidence of female gametes having taken part in fertilization with all numbers of chromosomes from 12 to 24, with exception of the numbers 19, 23, and 24. There is little

doubt that evidence would have been found for these missing numbers if more sections had been examined. The fact that over 2 per cent tetraploids were identified in the offspring of selfed triploids¹ is genetic evidence for functional female gametes with 24 chromosomes. In all cases in which counts were made in endosperm, there was a proembryo present.

The division of the fertilized egg in $2n$ *Datura* begins considerably later than that of the fertilized central nucleus which forms the endosperm.⁵ The same is true in $3n$ plants. Examination of serial sections through ovaries fixed 6–8 days after pollination in $3n \times 2n$ crosses showed some variability in the speed of development and in the behavior of ovules in the same ovary. In some ovules the development of the proembryos proceeded rapidly. They contained about 16–20 cells, and the endosperms had over 300 cells. In other ovules the development was slower. The proembryo contained 8–10 cells, or even fewer (2–6), and the number of cells in the endosperm was also smaller. In a number of ovules the fertilized eggs had not begun to divide, though the presence of empty pollen tubes in the micropylar end of the ovules and the formation of endosperm suggested that fertilization had taken place. A very large number of ovules contained unfertilized female gametes and central nuclei, and some of these ovules showed various stages of disintegration. Disintegration was also observed in some of the ovules in which fertilization had taken place.

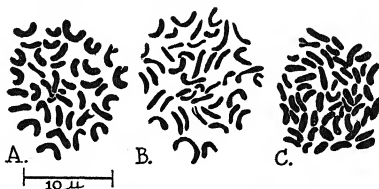


Fig. 2. Chromosomes in endosperm from cross $3n \times 2n$ (6 days after pollination): A. 38 chromosomes; B. 44 chromosomes; C. 54 chromosomes.

Counts made in serial sections through one whole ovary, which had been fixed 6 days after pollination, showed only 85 ovules with both proembryo and endosperm (60 proembryos had 2–4 cells, 24 proembryos had 5–6 cells, and one had 10 cells). Thirty-nine ovules showed fertilized eggs which had not begun to divide and endosperms with small numbers of cells. It is possible that most of these would have disintegrated at early stages of development. In one of the ovules of this series the proembryo seemed to be in good condition, but resorption had already begun in the endosperm, and in three other ovules the reverse condition was seen—the proembryo had disintegrated while the endosperm continued to divide. There remained several hundred ovules in this sectioned ovary, which were either unfertilized or showed more or less

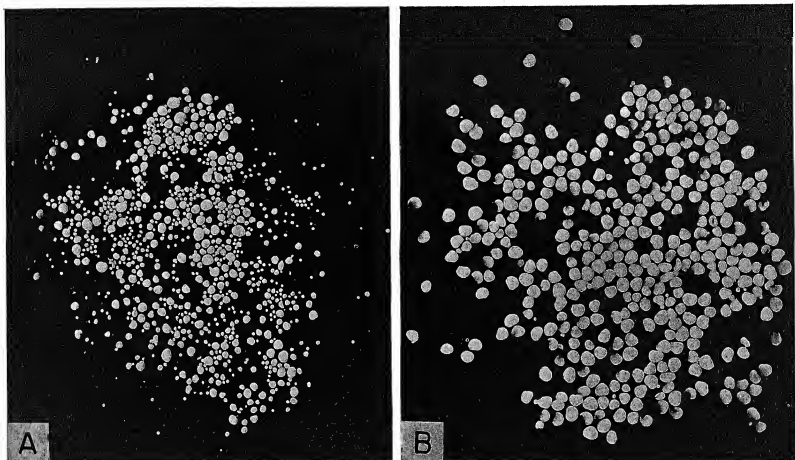


Fig. 3. Ovules from capsules 7 days after pollination. $\times 2$.—A. From the cross $3n \times 2n$.—B. From the cross $2n \times 2n$.

pronounced disintegration. In a few cases the division in the endosperm was obviously abnormal. In these the nuclei divided, but no cell wall developed between the daughter nuclei, and thus giant nuclei with double or quadruple numbers of chromosomes were formed. Such endosperms had few cells and obviously would have soon disintegrated.

Beside the counts of fertilized ovules made in the serial sections, another attempt to learn the approximate number of fertilized gametes was made by dissecting 5 ovaries which were fixed 7 days after a $3n \times 2n$ pollination. These ovaries contained 4125 ovules which is an average of 825 ovules per capsule (511, 793, 815, 895, 1111). According to their sizes the ovules were divided roughly into two groups. The group of small ovules included those between $\frac{1}{8}$ and $\frac{1}{4}$ mm. in length and probably represented ovules which either had not been fertilized or which had failed to develop after fertilization. The group of larger ovules included those from $\frac{1}{4}$ to $\frac{1}{2}$ mm. in length and represented ovules which had grown after fertilization. The larger ones of this group would have developed into seeds. Of the 4125 ovules from the $3n \times 2n$ cross, 3254 or 78.9 per cent were small, and 871 or 21.1 per cent were larger. Of 4066 ovules from 5 comparable capsules from a $2n \times 2n$ cross the relations were reversed. There were only 526 ovules, or 12.9 per cent, in the small group and 3540, or 87.1 per cent, in the larger group. The difference between the size of ovules from $3n \times 2n$ and from $2n \times 2n$ capsules of comparable age is shown in figure 3. Only a portion of the ovules from the $2n$ capsule is shown in figure 3B. The total number of ovules in a $2n$

and in a $3n$ capsule is approximately the same. The average number for our five $2n$ and $3n$ capsules is respectively 813 and 825. The smaller size of mature $3n$ capsules may be attributed to the fewer number of their ovules which develop into seeds. One can conclude from the observations on the $3n \times 2n$ crosses that though the extra chromosomes in the female gametes do not necessarily prevent fertilization, comparatively few ovules in $3n$ ovaries become fertilized. Furthermore, some of the fertilized ovules disintegrate at various stages of development.

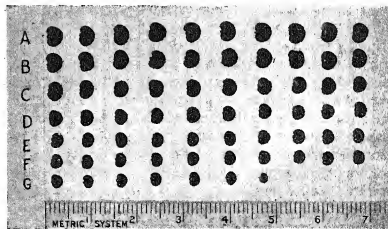


Fig. 4. Entire seed content of a single $3n \times 2n$ capsule.

SIZE, NUMBER, AND CONDITION OF SEEDS.—Mature seeds from $3n \times 2n$ crosses are very variable in size and number. In 75 capsules there were counted a total of 4588 seeds, which is an average of 61 seeds per capsule. The largest number found in one capsule was 136, the smallest number 23. In size they

ranged from 1.7 to 4.9 mm. in length. The length of one thousand seeds taken from 15 capsules was measured, and the seeds of each capsule were arranged according to sizes in descending order, as shown in figure 4. The capsule represented by this figure had 67 seeds; rows A-C2 contain the larger seeds (4.7-4.0 mm. long); rows C3-G7, the smaller seeds (3.9-2.3 mm. long). Table 1 gives a summary of the 1000 measurements made. A majority of the seeds (523) belong to the group 3.9-3.0 mm. long. A third (333) are large (4.9-4.0 mm.), and 144 are very small (2.9 mm. or less).

TABLE 1. Size and condition of seeds of $3n$ *Datura*.

Length in mm.	Totals	4.9-4.0		3.9-3.0		2.9-2.0		1.9-1.7	
		Number	%	Number	%	Number	%	Number	%
Good seeds ..	388	271	81.4	115	22.0	2	1.4	0	—
Defective seeds	120	35	10.5	83	15.9	2	1.4	0	—
Empty seeds	492	27	8.1	325	62.1	134	97.2	6	100.0
Totals	1000	333	33.3	523	52.3	138	13.8	6	0.6

A modification of Veh's method⁶ which decreased the loss of seedlings was used in germination of seeds. The coats were removed from the soaked seeds, which were left submerged in shallow water until germination started when they were transferred to soil. Removal of seed coats enabled one to learn the condition of the seeds tested. The use of water instead of soil afforded a better opportunity to record the day upon which germination started and to observe the speed of development for the first two to three days after germination. From the appearance of the contents after the seeds were opened, it was possible to classify them into three groups—good, defective, and empty. Those called good were plump, of normal shape, and with apparently good endosperm. Those called defective were characterized by various obvious abnormalities such as misshapen embryo or deficiencies in amount of endosperm. Types of defective seeds are shown in figure 6. Table 1 shows the size range of good, defective, and empty seeds in the 1000 seeds measured. Of these 38.8 per cent were good, 12 per cent were defective, and almost half (49.2 per cent) had nothing but seed coats. Most of the latter were found among seeds 3.9-1.7 mm. long, but seed coats only were also present in 8.1 per cent of the group of large seeds.

It will be noted from the table that the good seeds average largest, the defective seeds next in size, and the empty seeds smallest, but large and small seeds are found in all three groups. Since defective and empty seeds are rare in $2n$ capsules, their high proportion in $3n$ capsules is undoubtedly due to the unbalance of extra chromosomes.

Studies of the development of the integument in the ovule have been made by Souèges.⁹ Its protective, nutritive, and digestive functions during the

maturation of the seeds have been described in detail and require no further discussion here. The development of the proembryo in diploid *Datura* was also studied by Souèges.^{7,8} He found so much variability in the orientation of the cell walls at the earliest stages of the proembryo that it was impossible to predict which initial cell would give rise to particular tissues. A similar condition was found to hold true for the proembryos in the $3n \times 2n$ crosses. Later stages of normal development are given in figure 5A-J. Transverse hand sections made through seeds fixed 10-40 days after pollination show the relative

size of the integument, of the endosperm, and of embryo at various stages. One will observe the gradual decrease in size of the integument, the change in size and shape of the endosperm and of the embryo, and the time required for the differentiation of the radicle, cotyledons, and other parts of the embryo.

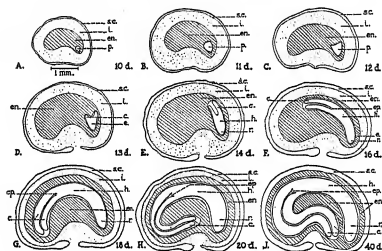


Fig. 5. Stages in normal development of seeds from $3n \times 2n$ capsule. *c*, cotyledon; *e*, embryo; *en*, endosperm; *ep*, epicotyl; *h*, hypocotyl; *i*, integument; *p*, proembryo; *r*, radicle; *s.c.*, seed coat. Time after pollination in days (*d*) is given for each ovule.

It has already been shown that there is a variability in the speed of development of fertilized ovules at early stages in a $3n \times 2n$ cross. The same has also been found true for later stages, 10-22 days after pollination. Most of the seeds fixed 10 days after pollination, however, had small, more or less spherical proembryos. The differentiation of cotyledons and of radicles could be easily seen under low power on the 12th to 14th day after pollination. The epi-

TABLE 2. Germination of good and defective seeds. (Average length of seeds in mm. shown in parentheses.)

Seeds	Total	Ungerminated seeds		Plants died		Plants recorded		2n		2n+1		2n+1+1		2n+1+1+1	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Good ...	388 (4.1)	11 (3.8)	2.8	106 (4.0)	27.3	271 (4.1)	69.8	57 (4.4)	21.0	134 (4.2)	49.4	71 (3.9)	26.2	9 (3.9)	3.3
Defective	48 (3.6)	24 (3.5)	50.0	10 (3.6)	20.8	14 (3.7)	29.2	1 (4.5)	7.1	4 (4.0)	28.6	8 (3.4)	57.1	1 (3.5)	7.1
Total ...	436 (4.0)	35 (3.6)	8.0	116 (4.0)	26.6	285 (4.1)	65.4	58 (4.4)	20.4	138 (4.2)	48.4	79 (3.9)	27.7	10 (3.9)	3.5

cotyl appeared after 15-16 days, and it took about 3 weeks for the embryo to reach full length. It takes over 3 weeks more for the seed to become mature. Comparison of developmental stages of normal seeds shown in figure 5A-J with the defective seeds shown in figure 6A-J will help one to understand better what had happened in some defective seeds. The size of seeds used for these drawings ranges from 3.2 mm. to 4.5 mm. in length. Some of them whether large or small had nothing but a small undifferentiated body under the seed coat (fig. 6A). Other seeds were more advanced when their growth had been arrested (fig. 6D). In some seeds a defective or a normal endosperm was found with no traces of embryo (fig. 6B, C); or vice versa, the endosperm was entirely or partially absent, but there was an embryo in which the cotyledons, hypocotyl, epicotyl, and radicle were evident (fig. 6E, F). Defective nutrition apparently arrested their further growth approximately 15-17 days after pollination. Some seeds showed a decayed condition of radicle, cotyledons, or hypocotyl (fig. 6J). In others the parts of the embryo were misplaced (fig. 6H). An extreme defect is seen in figure 6G, in which both the endosperm and the embryo were misshapen and rudimentary. The seed was only 3.2 mm. long, and a large portion of the integument with cells filled with starch remained undisturbed.

GERMINATION.—A low percentage of germination is characteristic for $3n \times 2n$ seeds of *Datura*, as also of other plants.² A total of 436 seeds were tested for germination. They comprised the 388 good seeds found among the 1000 seeds measured and 48 of the least defective from the 120 listed as defective seeds. After their seed coats had been removed, they were placed in Syracuse watch glasses with tap water, one seed in each, and kept on a desk at room temperature, in indirect light.

The results obtained are given in table 2. Only 11 (2.8 per cent) of the good seeds failed to germinate. One of them is still dormant, though it has been kept alternately in water and soil without germinating for over 14 months. During this period 10 seeds were destroyed by bacteria and molds. Of the 48 defective seeds, 22 were destroyed by bacteria, and 2 seeds which have been kept in water or soil for over 14 months have still failed to germinate.

The average size of the 11 good ungerminated seeds is 3.8 mm., that of the 24 ungerminated defective seeds is 3.5 mm. (table 2). The average size of 377 good seeds which germinated is 4.1 mm., and that of the defective seeds which germinated is 3.6 mm., but there is no great difference in the range in size of seeds between those which germinated and those which did not. It can be concluded that there is no direct relation between the size of the seed and its ability to germinate.

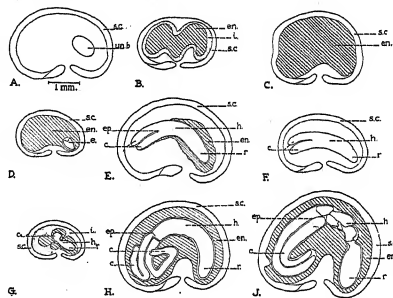


FIG. 6. Types of defective seeds taken from mature $3n \times 2n$ capsules: A. Undifferentiated body; B. Lobed endosperm, no embryo; C. Normal endosperm, no embryo; D. Normal endosperm, rudimentary embryo; E. Rudimentary endosperm, growth of embryo arrested; F. No endosperm, growth of embryo arrested; G. Endosperm and single cotyledon, both rudimentary; H. Endosperm normal, cotyledons misplaced; I. Hypocotyl in fragments disintegrating; J. Undifferentiated body; other abbreviations as in figure 5.

Though much care was taken to keep alive each of the 401 seedlings obtained, 116 plants were lost at various stages of development, and only 285 plants (65.4 per cent) reached a stage at which they could be recorded for type (table 2). Over half of the loss was apparently due to poor viability. The records show that 71 seedlings which grew extremely slowly died within a few days after germination, 7 plants died in advanced stages before records could be taken,

15 seedlings had decayed cotyledons, epicotyl, or radicle, and 23 plants died from accidents.

Data in table 2 show that the average size of the seeds which developed into mature plants and of those which died before the records could be made is almost the same. It is shown again that there is no direct relation between the viability and the size of the seed.

The average size of the seeds which developed into $2n$ plants is larger than that of seeds with one or more extra chromosomes (table 2) and the average size of $2n + 1$ seeds is larger than that of seeds with more than one extra chromosome. From table 3 it will be seen that in some cases there is a definite relation between the size of the seed and the particular extra chromosome present in the seed. Thus the seeds are smaller when the $1 \cdot 2$ or $15 \cdot 16$ (and possibly the $13 \cdot 14$) chromosome is present as an extra. The seeds are very large when the extra is the $11 \cdot 12$ and especially when the extra is the $23 \cdot 24$ chromosome. Seeds of these latter types are even larger than the $2n$ seeds (table 2).

To see whether the size of seeds could be used as a criterion for the type of offspring which will develop

Of the 116 seedlings which died early, 103 germinated within 3 weeks.

From the 285 offspring recorded, 234 plants (82.1 per cent) germinated in less than 3 weeks, and 51 plants (17.9 per cent) had delayed germination (table 3). Of the $2n$ plants all 58 germinated without delay in 3-12 days. Delayed germination was found among the $2n + 1$ plants (22.5 per cent, the $2n + 1 + 1$ plants (21.5 per cent) and the $2n + 1 + 1 + 1$ plants (30.0 per cent) (table 3).

It appears that certain of the chromosomes in the trisomes are in some way connected with the speed of germination or the length of dormancy of the seed. In all seeds with the $1 \cdot 2$ or the $21 \cdot 22$ extra chromosomes, whether single or in combinations with other extra chromosomes, there was no delay in germination (table 3). But records show that among the 18 seeds with the $3 \cdot 4$ chromosome extra, only 6 germinated early, while in 12 seeds the germination was delayed for more than 100 days. Furthermore, when the $3 \cdot 4$ chromosome was present as an extra in a $2n + 1 + 1$ seed, only 5 seeds out of 14 germinated early, while 9 seeds showed a dormancy averaging 109 days. In the single case in which the

TABLE 3. Speed of germination and size of seed with particular extra chromosomes.
N—normal germination (3-17 days); D—delayed germination (35-295 days).

Extra chromosome ...	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	Total
Average length	3.9	4.1	4.2	4.3	4.2	4.4	4.0	3.9	4.3	—	4.1	4.5	4.2
Germination	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D	N D
$2n$													58 0
$2n+1$	8 0	6 12	12 1	6 1	18 0	13 0	11 6	6 8	3 1	0 0	11 0	13 2	107 31
$2n+1+1$	6 0	5 9	20 2	1 3	17 4	13 2	23 10	11 3	1 0	1 0	10 0	16 1	62 17
$2n+1+1+1$	0 0	0 1	3 2	0 0	3 0	1 0	6 3	1 0	0 0	0 0	2 0	5 3	7 3
Total	14 0	11 22	25 5	7 4	38 4	27 2	40 19	18 11	4 1	1 0	23 0	34 6	234 51

from them, a preliminary test was made. It is known that the percentage of $1 \cdot 2$ offspring from seeds taken from $2n + 1 \cdot 2$ plants is not high, about 12 per cent. This percentage was increased to 69.2 per cent by making a rough selection of 60 of the smallest seeds from a capsule of a $2n + 1 \cdot 2$ plant.

The speed of germination of the seeds varied from 3-258 days. Records for the 401 seeds which germinated show that 337 of them (84.0 per cent) germinated within 3 weeks, and that in 64 (16.0 per cent) the germination was delayed for a considerably longer period. No direct relation was found between the speed of germination and the size of the seed. Some of the smallest seeds of a capsule were the first to germinate and some of the largest seeds were among the last, and vice versa. The same was found true for the defective seeds, in that some germinated early, others late. No direct relation could be traced between the poor viability of the seedling and the length of dormancy of the seed from which it came.

$3 \cdot 4$ was present as an extra in a $2n + 1 + 1 + 1$ plant, the seed germinated in 104 days. In total, 11 seeds with an extra $3 \cdot 4$ chromosome germinated early, and 22 seeds germinated with a considerable delay. It appears that the $13 \cdot 14$, $15 \cdot 16$, and the $7 \cdot 8$ chromosomes tend to bring about delayed germination when present as extras, though the influence is less marked than with the $3 \cdot 4$ chromosome. It may be significant, moreover, that in all cases but one when delayed germination was recorded in a $2n + 1 + 1$ or a $2n + 1 + 1 + 1$ seed, either the $13 \cdot 14$, the $15 \cdot 16$, or the $3 \cdot 4$, (or a combination of these chromosomes) was found as an extra in the offspring (19:1). Two seeds which germinated after $8\frac{1}{2}$ months gave weak plants which died after six weeks. They both were good seeds, their size 4.0 and 3.9 mm. The first of them had undoubtedly the $7 \cdot 8$ chromosome as an extra either alone or in combination.

TABLE 4. Increase of $1n$ nuclei in embryo sacs and in female gametes which take part in formation of recordable offspring.

Chromosome numbers	12	13	14	15	16	17	18	19	20	21	22	23	24
	Percentages												
(a) Calc. on random assortment	0.025	0.3	1.6	5.4	12.1	19.3	22.6	19.3	12.1	5.4	1.6	0.3	0.025
(b) Embryo sac	7.0	9.0	5.0	13.0	17.0	14.0	13.0	11.0	4.0	3.0	2.0	1.0	1.0
(c) ♀ gametes forming recordable offspring	20.0	48.4	27.7	3.5	0	0	0	0	0	0	0	0	0
(d) % in embryo sac % calculated	280.0	30.0	3.1	2.4	1.4	0.7	0.6	0.6	0.3	0.6	1.3	3.3	40.0
(e) % in offspring % calculated	816.0	161.3	17.3	0.65	0	0	0	0	0	0	0	0	0

It has been shown in an earlier paper¹ that 53.2 per cent of the offspring recorded from seeds of $3n \times 2n$ crosses were $2n + 1$ plants, 28 per cent were $2n$ plants, 16.6 per cent were $2n + 1 + 1$ plants, and 1.4 per cent were indeterminate.

Records of 285 plants made in the present paper, all but 12 of which were checked cytologically, show again that the largest number of individuals (48.4 per cent) had $2n + 1$ chromosomes. There were 20.4 per cent of $2n$ type, 27.7 per cent of $2n + 1 + 1$ plants, and 3.5 per cent of $2n + 1 + 1 + 1$ plants (table 2). The individual attention and care which was given to each plant of the triploid offspring in the present study enabled us to nurse along a few weak $2n + 1 + 1 + 1$ plants to a recordable age. This care of individual seedlings, the sowing of small and defective seeds, and the use of seeds with delayed germination explain why a higher percentage of plants with 2 and 3 extra chromosomes was recorded in the present than in the earlier study.¹ Some differences are evident in the two studies in respect to proportions of the different $2n + 1$ types. As can be seen from table 3, the most frequent $2n + 1$ types were the $2n + 9 \cdot 10$ and the $2n + 3 \cdot 4$ (18 cases each). The smallest frequency (4) was found for the $2n + 17 \cdot 18$. No $2n + 19 \cdot 20$ plants were recorded among the $3n$ offspring. The $13 \cdot 14$ chromosome was the most frequent as an extra in combination with others in the $2n + 1 + 1$ offspring (33 cases). It was the only chromosome which in this series was found combined as an extra with each of the other 11 chromosomes. The $19 \cdot 20$, $17 \cdot 18$, and $7 \cdot 8$ chromosomes were the least frequent in combinations with others in the $2n + 1 + 1$ seeds (1, 1, and 4 cases).

From table 2 it will be observed that a large majority of plants from defective seeds had extra chromosomes, only 1 of the 14 being $2n$. The most frequent type was $2n + 1 + 1$, whereas the most frequent type from good seeds was $2n + 1$. The average size of these seeds was smaller than that of good seeds. The speed of germination was in 8 cases normal and in 6 cases delayed over 100 days.

In a paper⁴ in the present series it was shown that, due chiefly to lagging chromosomes, there are 280 times as many $1n$ female gametes formed in $3n$ *Daturas* as would be expected from random assortment of chromosomes. The present paper shows that the proportions of the various types of gametes which take part in formation of offspring which live to a recordable age are further affected by differential elimination of the more highly unbalanced zygotes. This elimination is brought about through inability to form good seeds, through delayed germination, and through poor viability of seedlings. In table 4 are summarized the changes in frequencies of gametes with different chromosome numbers in the female $3n$ *Datura* in comparison with the frequencies calculated on random assortment. Eliminations in the zygotes bring about an increase of the frequencies of the $1n$, $1n + 1$, and $1n + 1 + 1$ classes of effective gametes over the same classes in the embryo sacs. A corresponding decrease is brought about in $1n + 1 + 1 + 1$ gametes and in those with a larger number of chromosomes. A similar relation is found to hold if the comparison is made between the effective gametes and the types expected from random assortment. If attention is centered on the $1n$ gametes only, there are about 3 times as many $1n$ gametes that take part in the promotion of fully viable offspring as one would expect from the counts in embryo sacs and 816 times as many as one would expect from random assortment.

It is not clear why $3n$ types have not appeared in the offspring from the cross $3n \times 2n$, neither in the present series nor in extensive plantings from crosses between triploids and gene types in experiments for the purpose of locating genes by trisomic ratios. The fact that over 2 per cent $4n$ individuals have been found in offspring from selfing $3n$ parents proves that the $2n$ female gametes may take part in formation of viable zygotes. We know of no reason why there should be selection against $3n$ zygotes. That they may be at a disadvantage in comparison with $2n$ and $4n$ zygotes, however, is suggested from the results of the cross $4n \times 2n$, which gives relatively few seeds, and these have poor germination. In a single series

of $4n \times 2n$ crosses in which the seeds were counted there were 10 capsules and 64 large seeds and a considerable number of small, probably empty, seeds. From the 64 seeds, only 4 germinated. Three of the seedlings were $3n$, and one was $4n$, which latter may have been the result of accidental selfing.

The present paper on the seed and the former two papers respectively on the male and the female gametophytes discuss the factors effective at the different stages of development in the elimination of highly unbalanced gametes and zygotes and the consequent relatively large numbers of $2n$ individuals in the offspring of $3n$ *Daturas*.

SUMMARY

Following $3n \times 2n$ pollinations, fertilization may occur in ovules with female gametes which have chromosome numbers from 12 to 24. The majority of the female gametes remain unfertilized. In many fertilized ovules the proembryo and endosperm disintegrate soon after fertilization. Due to arrest of growth at later stages, nearly half the seeds in the adult capsule are empty, about an eighth are defective, because of absence or partial development of embryo or endosperm, leaving only slightly more than a third which are normal in appearance. Elimination of female gametes and of zygotes at various stages of development reduces the number of seeds in a $3n \times 2n$ capsule to an average of about 61 against an average of about ten times as many for compar-

able $2n \times 2n$ capsules. There is a considerable range in size of seeds in $3n$ capsules, but no close relationship was evident between size and either germination or viability of seedlings. The $2n$ seeds average larger than the $2n + 1$ seeds, and the latter average larger than the $2n + 1 + 1$ seeds. Certain chromosomes when present as extras in $2n + 1$ embryos caused the seeds to be larger, and certain other chromosomes caused the seeds to be smaller. The $2n$ seeds germinated promptly within 3 to 12 days, while germination was considerably delayed in some of the seeds with extra chromosomes.

The proportion of seeds with delayed germination was highest when the 3.4, the 13.14, or the 15.16 chromosome was the extra. The present study shows that zygotes with extra chromosomes tend to be differentially eliminated in comparison with $2n$ zygotes through inability to develop good seeds, through delayed germination, and through poor viability of seedlings. These factors, together with the eliminations in male and female gametophytes discussed in earlier papers, are responsible for the relatively large number of $2n$ individuals in the offspring of $3n$ *Daturas*, a number 816 times that expected on random assortment of chromosomes.

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STUDIES ON THE VIRUS OF TOBACCO NECROSIS¹

W. C. Price

AN INTERESTING virus disease of tobacco was described in 1935 by Smith and Bald, who gave it the name tobacco necrosis. While the disease has proved to be of little economic importance, it is of considerable scientific interest because the virus concerned is present in roots of otherwise normal plants, is highly resistant to aging, heat, and chemical agents, and is reported to be both water- and air-borne. The virus thus appears to be an unusual one—so unusual, in fact, that Smith (1935) suggested the possibility of its arising *de novo* in order to explain some of the results obtained. Because of the apparently unusual behavior of the virus, it was studied in the hope of gaining further information regarding its specific relationships with other viruses, some of its properties, and its modes of transmission. While the results obtained agree in general with those reported by Smith and Bald (1935) and by Smith (1937a, 1937b), they differ in several respects and lead to the general conclusion that tobacco-necrosis virus is not fundamentally different from other plant viruses. It is the purpose of this paper to present the results of the studies and to point out some of the significant characteristics of the virus.

SYMPTOMATOLOGY.—Tobacco necrosis has a wide host range, infecting plants in at least 10 different families. Since the symptoms produced in these plants have been described by others (Smith and Bald, 1935; Smith, 1937a, 1937b), only a brief review need be given here. The most characteristic feature of the disease is the almost complete localization of symptoms in all the known host plants. Inoculated leaves of tobacco develop a large number of necrotic lesions (fig. 1) which later enlarge, fuse, and in time may cause necrosis of the entire leaf. If, however, the inoculum is dilute and only a few lesions develop, they usually appear zonate, enlarging gradually to produce concentric rings of alternating green and necrotic tissue. Occasionally, lesions of similar type may appear on leaves above the inoculated ones, but these are few in number, occur rarely, and are always on leaves well below the growing point. During the past winter, many infected tobacco plants were under observation, but none of them developed systemic symptoms. In general, the symptoms on most of the hosts of tobacco necrosis resemble those produced in tobacco. The writer has not observed the disease occurring naturally on any host plant, but Smith and Bald (1935) report the frequent occurrence of natural infection in White Burley tobacco seedlings with the production of necrotic lesions on the young leaves and occasionally necrosis of the stem and growing tip, resulting in death of the seedling so affected.

In the course of an experiment designed for another purpose, necrotic lesions were observed in tobacco plants within 23 hours after they had been inoculated.

¹ Received for publication July 5, 1938.

Since no other plant virus has been observed to cause symptoms in less than about 36 hours, it was of considerable interest to determine the shortest time in which symptoms of tobacco necrosis could be detected. A group of young Turkish tobacco plants was divided into lots of 5 plants each and inoculated heavily at hourly intervals throughout the day. On the following day, which proved to be cloudy, the plants were observed for first appearance of symptoms. Plants inoculated in the morning required a longer time to develop symptoms than those inocu-



Fig. 1. Necrotic primary lesions produced in a leaf of Turkish tobacco by tobacco-necrosis virus. The photograph was taken 10 days after inoculation. (Photograph by J. A. Carlile.)

lated in the afternoon. The shortest time required was 21½ hours in plants inoculated at 5:30 p.m. and the longest time was 26 hours in plants inoculated at 9:30 a.m. Symptoms first appeared as small yellow lesions not more than 0.2 mm. in diameter. The lesions gradually enlarged during the day until they reached a diameter of about 1.0 mm. They did not become necrotic until about 30 hours after inoculation. In a repetition of this experiment, made on a

more sunny day, similar results were obtained except that symptoms appeared several hours earlier and were necrotic when first observed. In this experiment the shortest time required for development of symptoms was 18 hours in plants inoculated in late afternoon and the longest time 24 hours in plants inoculated in the morning.

Production of necrotic lesions by tobacco-mosaic virus in *Nicotiana glutinosa* L. in about 36 hours (Holmes, 1930) was considered remarkable at the time it was first reported. It was, therefore, astonishing to find that tobacco-necrosis symptoms require only about one-half that time to become evident. No other plant virus is known to produce symptoms in such a short time.

In the tests to be reported, ordinary tap water was used as a diluent. Virus was extracted from tobacco leaves inoculated from 3 to 5 days previously and made up into 8 different dilutions. The 8 dilutions were paired in all possible ways and each pair was tested on opposite leaves of cowpeas. The method of randomization followed was similar to that described by Youden (1937) as incomplete blocks with the two primary leaves on a plant considered as a block. After 3 days the lesions were counted and tabulated. The data from 4 tests are given in table 1. When the data for each test were plotted with the logarithm of the dilution as the X-axis and the logarithm of number of lesions as the Y-axis, the points obtained were found to lie along a straight line. The

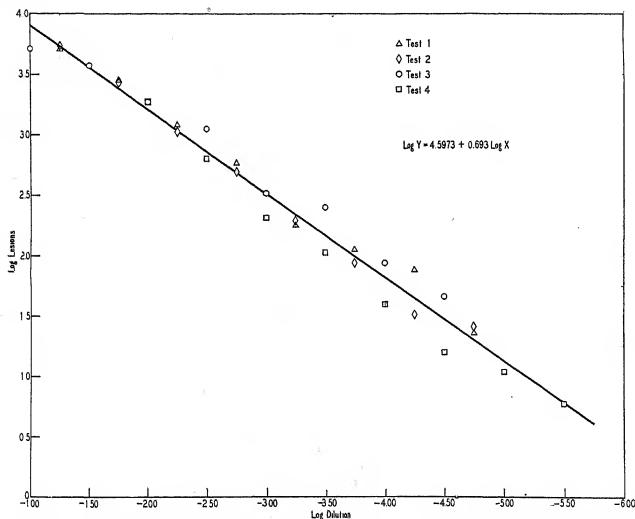


Fig. 2. Dilution curve for tobacco-necrosis virus as indicated by numbers of necrotic lesions produced in cowpea leaves.

PROPERTIES OF THE VIRUS.—*The dilution curve.*—Lesions produced by tobacco-necrosis virus on the Black variety of cowpea (*Vigna sinensis* Endl.) are uniform solid necrotic spots, easily counted, and hence well adapted for use in measuring virus concentration. Moreover, the cowpea may be grown from seed to the stage at which it is suitable for inoculation in about 9 or 10 days, whereas some of the other susceptible species, such as tobacco, require a much longer time. For these reasons, the Black variety of cowpea was chosen as a host plant for dilution curve studies and has subsequently been used for measuring virus concentration.

equations for the 4 tests were calculated and found to be as follows:

$$\text{Test 1. } \text{Log } Y = 4.066 + 0.66 \log X$$

$$\text{Test 2. } \text{Log } Y = 4.083 + 0.70 \log X$$

$$\text{Test 3. } \text{Log } Y = 4.477 + 0.61 \log X$$

$$\text{Test 4. } \text{Log } Y = 4.204 + 0.72 \log X$$

From these equations it can be seen that, while the actual virus concentration differed somewhat in the 4 tests, the slopes of the curves are similar, varying between 0.61 and 0.72. From the data of table 1 it seems apparent that the virus used in test 3 was about 6 times as concentrated as that used in tests

1 and 2. This difference is not surprising when it is considered that inoculated leaves were used as a source of virus and that the virus concentration of such leaves is determined to a large extent by the numbers of lesions produced in them. If allowances are made for the differences in concentration in the 4 tests and the data plotted accordingly, the graph shown in figure 2 is obtained. The points are found to lie reasonably close to a straight line with a slope of about 0.7.

TABLE 1. Number of necrotic lesions produced in 28 leaves of cowpea with virus samples of different dilutions.

Logarithm of dilution	Test number			
	1	2	3	4
-0.5	5217	5264	—	—
-1.0	2833	2758	5229	—
-1.5	1194	1030	3732	1838
-2.0	587	501	1862	634
-2.5	182	197	1116	207
-3.0	113	88	327	106
-3.5	77	33	252	40
-4.0	23	26	88	16
-4.5	—	—	46	11
-5.0	—	—	—	6

The high infectivity of tobacco-necrosis virus is of significance, particularly in view of the fact that the virus is localized in primary necrotic lesions. As shown in table 1 and figure 2, virus from such lesions has a dilution end point of about 10^{-6} and an infectivity, therefore, about $1/10$ as great as that of tobacco-mosaic virus in the juice of tobacco plants. It was of interest to compare the infectivity of tobacco-mosaic and tobacco-necrosis viruses secured

with saline. Each dilution of tobacco-mosaic virus was tested by inoculation of 15 leaves of *N. glutinosa*, while each dilution of tobacco-necrosis virus was tested by inoculation of 32 leaves of cowpea. The 15 *N. glutinosa* leaves were roughly of the same area as the 32 cowpea leaves. The numbers of lesions produced are given in table 2. The equations for the two sets of data were found to be as follows:

For tobacco-mosaic virus,

$$\log Y = 3.829 + 0.711 \log X.$$

For tobacco-necrosis virus,

$$\log Y = 4.333 + 0.822 \log X.$$

The difference between the two equations is no greater than that found previously between different tests with tobacco-necrosis virus and is not considered to be significant. It appears that the infectivity of tobacco-necrosis virus is as great as, if not greater than, that of tobacco-mosaic virus from a host plant in which both viruses are localized in necrotic lesions.

Thermal death point.—The thermal death point of the virus of tobacco necrosis has been reported by Smith and Bald (1935) to be from 70° to 72°C . In making their tests, these workers first passed the juice from diseased plants through a sand and paper pulp filter. It is probable that filtration not only removed extraneous matter from the juice but also reduced the virus content. Both of these things would be expected to lower the thermal death point of the virus. It was, therefore, not surprising to find that freshly expressed juice from diseased Turkish tobacco plants still remained infectious after heating to temperatures considerably above 72°C .

Leaves of Turkish tobacco plants that had been rubbed with an undiluted virus solution from 4 to 6 days previously were ground in a sterile meat chopper. Juice was extracted from the pulp by passage through one thickness of cheese cloth and placed in

TABLE 2. Numbers of necrotic lesions produced in 32 leaves of cowpea and 15 leaves of *Nicotiana glutinosa* with different dilutions of tobacco-mosaic and tobacco-necrosis viruses, respectively, from the same species of host plant.

Virus	Logarithm of dilution											
	-0.5	-1.0	-1.5	-2.0	-2.5	-3.0	-3.5	-4.0	-4.5	-5.0	-5.5	-6.0
Tobacco mosaic ...	2400	1392	629	361	118	46	22	4	8	—	—	—
Tobacco necrosis ..	—	2991	1786	480	186	79	18	10	3	2	1	0

from a host plant in which they produce similar symptoms. Holmes' (1938) necrotic-type tobacco, derived from the *N. digluta* Clausen and Goodspeed \times *N. tabacum* L. hybrid, was chosen as a host plant, since it responds to infection with tobacco-mosaic virus as well as with tobacco-necrosis virus by the production of primary necrotic lesions. Leaves of this tobacco inoculated 3 days previously and showing comparable numbers of lesions were used as a source of virus and made up into several dilutions

7×70 mm. test tubes, 1 cc. per tube. The tubes were tightly corked and completely immersed in a constantly stirred electrically heated water bath held within 0.2°C . of the desired temperature. After remaining in the bath for 10 minutes, the tubes were removed and immediately plunged into cold water. Virus activity of the heated samples was measured by inoculation of 28 leaves of cowpea with the virus sample to be tested. The data obtained are shown in table 3.

TABLE 3. Number of lesions produced in 28 cowpea leaves by samples of tobacco-necrosis virus heated to different temperatures for 10 minutes.

Temperature	Test number		
	1	2	3
Controls	16033 ^a	30340 ^b	3400
80°C.	229	788	955
82°C.	117	483	490
84°C.	74	310	180
86°C.	26	114	35
88°C.	10	63	28
90°C.	0	14	6
92°C.	0	0	0
94°C.	—	—	0

^a Calculated from the number of lesions (3198) produced by a 10⁻¹ dilution of the virus sample.

^b Calculated from the number of lesions (1209) produced by a 10⁻² dilution of the virus sample.

From these data it is apparent that the thermal death point of tobacco-necrosis virus in freshly expressed juice of diseased tobacco plants is between 90° and 92°C., a temperature about 20° higher than that reported by Smith and Bald (1935). The discrepancy between the writer's results and those of Smith and Bald is not difficult to explain when it is realized that the unheated controls of the latter produced only 10 to 15 lesions per leaf, whereas those used in the present experiments gave about 300 per leaf (test 3 of table 3).

The usual procedure, in the past, for determining the thermal death point of plant viruses has been to heat fresh untreated juice of diseased plants. The data of table 2 may, therefore, be compared directly with those obtained for other viruses. They show that tobacco-necrosis virus has a thermal death point only slightly below that reported for tobacco-mosaic

with a pH of from 8 to 11 became somewhat more acid, while the others did not change appreciably. At the end of the 24-hour period, the samples were readjusted to pH 6.4 by addition of NaOH or HCl and tested on cowpea for infectivity. The data, presented in table 4, show that infectivity of the virus was not diminished at pH 3 to pH 9 inclusive, was decreased appreciably at pH 2 and pH 10, and was destroyed at pH 1 and pH 11. The large number of lesions produced by some of the treated virus samples as compared with the original juice (at pH 6.4) may have resulted from increased salt content of the solution.

Best and Samuel (1936) have suggested the use of a composite buffer solution for determining the effect of hydrogen-ion concentration on plant viruses. The buffer is prepared by making a solution 0.05 molar with respect to each of the following: boric acid (H₃BO₃), potassium dihydrogen phosphate (KH₂PO₄), and potassium hydrogen phthalate (KHC₈H₄O₄). Different aliquots of the buffer are then adjusted to the desired pH by addition of HCl or NaOH. The use of buffers has the advantage of maintaining the electrolyte content of solutions at all pH values approximately the same. Additional tests on effect of pH on tobacco-necrosis virus were made by using the buffers suggested by Best and Samuel adjusted to pH 1 to pH 12 in 1 pH gradations. In the first of these tests, juice extracted from diseased plants and clarified by filtration through celite (Hyflo Standard-cel) was added to the buffer solutions in the proportion of 1 part of virus to 9 parts of buffer. In the second test, a 10⁻¹ dilution of unclarified juice from diseased plants was added to the buffer solutions in the proportion of 1 to 9. As will be seen in table 5, the addition of juice altered the pH of the buffer except at the extremes of the pH range, most samples tending to approach the neutral point. The samples were tested immediately after adjustment in order to determine the effect of pH of the inoculum on num-

TABLE 4. Lesions produced in 32 leaves of cowpea by inoculation with virus samples adjusted to pH 6.4 after standing at different pH's for 24 hours.

pH →	1	2	3	4	5	6	7	8	9	10	11
Lesions →	0	4	1936	2624	2442	824	3098	2903	2012	489	1

virus (Price, 1933), about the same as that of cucumber viruses 3 and 4 of Ainsworth (1935), and considerably higher than that of most plant viruses.

Effect of hydrogen-ion concentration on infectivity.

Tests were made to determine the effect of hydrogen-ion concentration on the virus of tobacco necrosis. Juice was extracted from infected tobacco leaves and diluted 10⁻¹ with tap water. After dilution it was found to have a pH of 6.4. Different portions of the diluted juice, each about 50 cc., were adjusted to pH 1 to pH 11 in 1 pH gradations by addition of NaOH or HCl, and allowed to stand for 24 hours at room temperature. During this interval, the samples

were of lesions produced. The effect of pH on inactivation of the virus was determined by testing the samples after 24 hours when they were readjusted to about pH 7 by the addition of an equal volume of 0.1 molar potassium phosphate buffer. The data obtained are given in table 5 and are illustrated in figure 3. Lesion counts from samples tested immediately after adjustment increased with increase in pH of the solution up to about pH 10, no infection being obtained at pH 11.6. Lesion counts from samples readjusted to pH 7 show that the clarified and unclarified samples behaved somewhat differently with respect to inactivation. Both samples were ap-

parently most stable at a pH of about 4.5 or 5.0, their infectivity dropping off sharply at lower pH values. The infectivity of the clarified virus sample also dropped off sharply at pH values above pH 4.5, but that of the unclarified dropped only slightly.

of tobacco necrosis, under certain conditions at least, is fairly stable over a rather wide range in hydrogen-ion concentration. In this respect, the effect of hydrogen-ion concentration is similar to that reported by Stanley (1935) for tobacco-mosaic virus.

TABLE 5. Lesions produced in 32 leaves of cowpea with virus samples adjusted to different pH's with composite buffer solutions.

Clarified juice			Unclarified juice		
pH at which samples were held	Tested immediately at different pH's	Tested at pH 7 after standing 24 hrs. at different pH's	pH at which samples were held	Tested immediately at different pH's	Tested at pH 7 after standing 24 hrs. at different pH's
1.09	0	0	1.11	1	0
3.13	425	359	2.78	162	252
4.50	251	613	4.03	170	555
4.90	316	447	5.07	303	651
5.26	568	375	5.68	319	649
5.89	482	201	6.37	531	587
6.61	866	124	7.13	597	599
7.04	745	158	7.75	856	544
7.50	1242	117	8.60	1596	459
8.10	1984	120	9.33	1345	486
8.62	1990	65	9.72	2881	429
11.64	0	0	—	—	—

The data from the 3 experiments just described are difficult to interpret because of the wide variation between the different tests. It seems preferable, therefore, not to draw definite conclusions until it is possible to conduct experiments with purified samples of virus. However, it seems apparent that the virus

OCCURRENCE AND TRANSMISSION OF THE DISEASE.—*Test for virus in old plants.*—The report by Smith (1937a) that tobacco-necrosis virus is frequently found in roots of old plants grown in greenhouses in England suggested that the virus might also occur in roots of plants grown in this country. Accordingly,

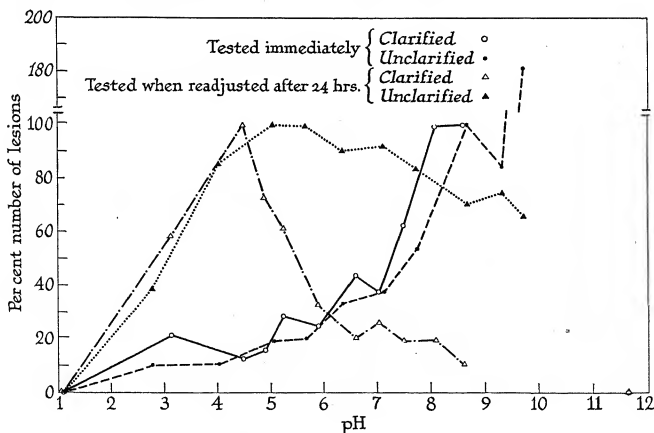


Fig. 3. Curves illustrating the per cent number of lesions produced with clarified and unclarified samples of tobacco-necrosis virus tested immediately after adjustment to different pH values, and tested 24 hours later when readjusted to about pH 7.

a search was made for virus in roots, stems, and leaves of the following old plants grown from seed in a greenhouse for this purpose: 7 Bonny Best tomato (*Lycopersicon esculentum* Mill.), 5 *N. rustica* L., 5 *N. langsdorffii* Weinm., 56 Burley tobacco (*N. tabacum*), and 8 Turkish tobacco (*N. tabacum*). The portion of the plant to be tested was ground up and rubbed, by means of a cheese cloth pad, over the leaves of two or more young Turkish tobacco plants. In no case was virus recovered from either roots, stems, or leaves of any of the plants tested.

Dr. K. M. Smith then supplied the writer with some seed of White Burley tobacco. A test was made of the leaves, stems, and roots of 180 plants grown to a height of about 14 inches from this seed. No virus was found in 179 of these plants. On the other hand, the roots of one plant gave infection when ground up and rubbed over the leaves of healthy Turkish tobacco plants. The virus was transferred

concluded that the virus could infrequently infect susceptible plants by passage through stomata. Because of the importance of determining whether or not the tobacco-necrosis virus may gain entrance to the cells of healthy plants by means other than through injured tissue, it was considered of interest to conduct spraying experiments with this virus. A 1:10 dilution of juice containing tobacco-necrosis virus was sprayed, by means of an atomizer, over the leaves of several different species of susceptible plants. The plants were afterwards separated into two approximately equal groups, the leaves of one group being rubbed with a finger tip or cheese cloth pad, those of the other group not being rubbed. They were placed on a greenhouse bench and exposed to the usual greenhouse treatment. After 4 days, counts were made of the lesions produced on rubbed and unrubbed plants. The data obtained are given in table 6.

TABLE 6. Numbers of necrotic primary lesions produced in different plants sprayed with a suspension of tobacco-necrosis virus.

Kind of plant	Rubbed		Not rubbed		
	No. of plants	Lesions	No. of plants	No. plants with lesions	Total no. lesions
<i>Nicotiana tabacum</i> L. (Turkish tobacco) .	4	2520	7	3	13
<i>Nicotiana glutinosa</i> L.	6	1250	6	1	1
<i>Nicotiana rustica</i> L.	1	100	1	0	0
<i>Phaseolus vulgaris</i> L.					
Early Golden Cluster bean	4	1000	4	0	0
Corbett Refugee bean	2	400	2	2	3
Resistant Great Northern bean	2	600	2	0	0
<i>Vigna sinensis</i> Endl. (Black cowpea)	15	9300	13	1	1

through a series of Turkish tobacco plants, and produced primary necrotic lesions in all cases but never became systemic. Unfortunately, the virus was lost before any experiments were made with it, and hence no accurate comparison is available between it and tobacco-necrosis virus. However, so far as tested, it behaved exactly like the latter and was undoubtedly that virus or a closely allied strain. The exact origin of the virus is obscure. There seems to be little doubt, however, that it was associated with the tobacco seed supplied by Dr. Smith, since the virus had not previously been found to occur in the greenhouses at Princeton. Whether the virus was carried in the seed or was merely adhering to the seed coat has not been determined.

Attempts to infect plants by spraying virus over their leaf surfaces.—It has been reported by Smith and Bald (1935) that plants may be infected merely by spraying virus over their leaves. About half the plants sprayed became infected but produced only a small number of lesions—i.e., 2 or 3 per leaf. These results are similar to those obtained with tobacco-mosaic virus by Duggar and Johnson (1933), who

While large numbers of lesions developed in those plants that were rubbed after being sprayed, only a few lesions were produced in the unrubbed ones. Many plants remained completely healthy. The somewhat larger number of lesions produced in unrubbed leaves of Turkish tobacco is believed to be due not to greater susceptibility of this host but to the fact that the plants were more compact and thus their leaves had a greater chance of rubbing together during natural growth movements. It seems worthy of mention in this connection that the tobacco plants used were trimmed before spraying to about 4 leaves each, and that in no instance did a lesion develop on the uppermost leaf of a plant. The lesions always appeared on leaves whose upper surfaces could have come into contact with the leaf above.

Although results of the nature just described do not rule out the possibility of stomatal infection, they may be interpreted in other ways. If stomatal infection does occur, it is hardly conceivable that a single plant should escape infection when exposed to large dosages of virus. It might be mentioned in this connection that Johnson (1936, 1937) failed to demon-

strate stomatal infection of a highly susceptible tobacco hybrid by spraying with tobacco-mosaic virus.

Transmission of the virus to roots.—Test for virus in roots of infected plants.—During the course of investigations on tobacco necrosis in Turkish tobacco plants, symptoms have been observed only rarely on portions other than those actually inoculated with the virus. It seemed possible, however, that the virus might move from the site of inoculation into other parts of the plant without producing symptoms. Tests were, therefore, made to determine whether or not virus could be detected in symptom-free portions of tobacco plants infected with the disease. The tops of many infected plants tested from time to time were, as previously reported by Smith and Bald (1935), always found to be virus-free. On the other hand, virus was found frequently in roots of infected plants. In one test, for example, the roots of 12 old Turkish tobacco plants having tobacco necrosis were tested and 9 of them found to carry virus. The presence of virus in such roots does not prove that the virus moved from the inoculated leaf down the stem and into the roots, since it is known that virus poured on the soil is capable of infecting roots growing therein (Smith, 1937b). On the contrary, evidence that the virus does not ordinarily move from the site of inoculation into the roots is afforded by the following experiment with 12 Turkish tobacco plants growing in 4-inch pots. The tops of the pots were covered by two thicknesses of wax paper, leaving a small hole for the plants to grow through. Leaves of 8 of the 12 plants were rubbed with virus of tobacco necrosis and afterwards washed in such a manner that none of the virus used for inoculation could be carried down into the soil. The remaining 4 plants were kept for controls. All the pots were placed in saucers and watered from the bottom only. After 47 days, roots of each of the 12 plants were tested for virus by inoculation of 4 plants of Black cowpea. None of the cowpea plants developed lesions, indicating that none of the tobacco roots were carrying virus at the time they were tested. The data indicate that under the usual greenhouse conditions, when precautions are taken to prevent accidental infection of roots, the virus of tobacco necrosis remains completely localized in tobacco and that it is probably confined to inoculated leaves. It is possible that under unusual conditions or in the case of other host plants the virus might become systemic.

Infection of roots.—It has been reported by Smith (1937b) that the virus of tobacco necrosis, when present in soil in small quantities, is able to infect the roots of healthy tobacco plants. An experiment was undertaken to determine the extent of root infection when virus is poured directly on the soil in pots containing healthy Turkish tobacco plants. Twenty-four young Turkish tobacco plants in 4-inch pots were divided into sets of 6 pots each. Ten cc. of a virus solution were poured on the soil in each pot in 3 of the sets, a 10^{-1} dilution being used for the first set, a 10^{-2} dilution for the second set, and a 10^{-3} dilution

for the third set. No virus was added to the soil in the fourth set. The pots were placed in saucers and kept on a greenhouse bench for a period of 3 weeks. At the end of this period the plants were examined for symptoms, but none whatever had developed. The roots were then removed from the soil, washed in tap water, ground up, and used to inoculate cowpea plants. The numbers of lesions produced on the different sets of cowpeas are recorded in table 7.

TABLE 7. *Numbers of lesions produced in 10 leaves of cowpeas with inocula from roots of Turkish tobacco plants.*

Plant no.	Quantity of virus used to inoculate roots			
	1.0 cc.	0.1 cc.	0.01 cc.	0.00 cc.
1	169	0	0	0
2	74	575	0	0
3	121	498	0	0
4	224	884	0	0
5	124	372	137	0
6	100	0	25	0

These data show that the roots of tobacco plants were infected with tobacco-necrosis virus when even as little as 0.01 cc. of the virus was added to the soil. Multiplication of the virus in the roots did not appear to be correlated with amount of virus used for inoculation. On the other hand, the number of plants infected was apparently affected by the concentration of virus used as inoculum. All of the plants inoculated with 1.0 cc. of virus, 4 of those inoculated with 0.1 cc., and only 2 of those inoculated with 0.01 cc. of virus became infected. It is possible, of course, that none of the plants would have escaped infection if they had been allowed to grow for a longer period before being tested.

While this experiment demonstrates that root infection does take place when virus is added to the soil, it yields no information regarding the mode of infection. Some indication of the mode of infection is afforded by the report of Smith (1937b) that the virus seems unable to enter the roots of cuttings grown in water culture and that soil or sand is, therefore, a necessary, though possibly only mechanical, agent in the process of root infection. In order to obtain further evidence on the question of whether wounding is necessary for root infection, plants were grown in nutrient solutions to which small quantities of virus were later added. Turkish tobacco seedlings were transferred to 250 cc. dark brown bottles containing KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, and MgSO_4 at an osmotic value of 0.5 atmosphere and containing a trace of FeSO_4 , H_3BO_3 , and MnSO_4 [R_2S_2 of the National Research Council series (Livingston, 1919)]. The bottles were kept full by addition of distilled water, the nutrient being changed once during the experiment. When the plants had developed a good-sized root system, they were divided into 3 lots of 6 each.

To the bottles containing the first 6 plants was added from 0.1 to 0.3 cc. of juice from diseased plants. Roots of the second lot of plants were rubbed with a cheese cloth pad moistened with diseased juice. The third set of plants was kept as controls. Three weeks later the roots were removed, thoroughly washed in tap water, and tested for virus by inoculation of Turkish tobacco and cowpea plants. Infection was obtained from all the roots that had been rubbed with virus solution. The remainder of the roots, with one exception, failed to give infection. In one case a few lesions were produced by roots that had been inoculated merely by addition of virus to the culture solution, but the number of lesions was so small (2 lesions on a total of 4 tobacco leaves) that they may have resulted from virus adhering to the root surface and not removed by washing.

On another occasion the experiment was repeated, using 16 tobacco plants for each treatment. The results were practically identical with those obtained previously. No infection was obtained in roots grown in a culture solution to which from 0.5 to 1.0 cc. of diseased juice had been added, but was obtained in 7 of the roots rubbed with virus. The results of the experiment indicate that roots do not ordinarily take up virus from the medium in which they are growing and suggest an explanation for infection of roots grown in soil. It is possible that young roots are injured as they push their way through the soil and that virus coming in contact with the injured portion causes infection. An alternative explanation is that the virus is transmitted to the roots by organisms living in the soil.

Infection of roots with tobacco-mosaic virus.—It would seem that tobacco-necrosis virus is an unusual one with respect to its ability to infect plants by entrance through their roots. On the other hand, McKinney (1923) has reported transmission of wheat-rosette, or mosaic, virus by means of contaminated soil, and it thus appears that other viruses might be similarly transmitted. It is known that the soil is a common source of primary infection for tobacco mosaic (McKinney, 1927; Lehman, 1934; Johnson, 1937). The exact site of infection in the case of soil transmission has not, however, been satisfactorily demonstrated. Allard (1917) reported that tobacco plants could be infected with mosaic disease by root inoculation. On the other hand, certain workers (Mylvania, 1930; Lehman, 1934; Johnson, 1937) have reported results indicating that tobacco roots are either immune or highly resistant to infection with tobacco-mosaic virus.

Because of the unsettled state of our knowledge concerning root infection, experiments were undertaken in order to determine whether or not tobacco-mosaic virus could be transmitted to the roots of healthy tobacco plants by addition of virus to the soil in which the plants were growing. Although preliminary experiments indicated that transmission did take place, there was doubt as to the actual point of infection. More carefully controlled experiments

were, therefore, made. Ten cc. of a 10^{-1} dilution of tobacco-mosaic virus were poured over the soil in each of 4 pots in which *N. glutinosa* plants were growing. *N. glutinosa* was used since it responds to infection by the production of necrotic lesions at the site of inoculation on stems or leaves. No evidence of stem or leaf infection was obtained on any of the 4 plants. After 3 weeks, the roots of each of the 4 plants were washed free of soil with tap water, ground up, and used to inoculate 5 leaves on a plant of *N. glutinosa*. A total of 29, 24, 22, and 10 lesions, respectively, were produced on the 4 test plants. No lesions were obtained when roots of *N. glutinosa* not exposed to tobacco-mosaic virus were tested in a similar manner.

In an experiment with Turkish tobacco, the soil in each of 6 pots containing young tobacco plants was covered with a layer of plaster of paris and the pots set into saucers each of which contained 10 cc. of a 10^{-1} dilution of tobacco-mosaic virus. It had previously been found that virus added in this manner would be taken up through the small hole in the bottom of the pot and could be detected in low concentration at least 4 days later in the soil near the top of the pot. None of the plants showed symptoms of infection during the 21 days they were kept under observation. At the end of this time, the roots were tested for virus as previously described by inoculation of 5 leaves on a plant of *N. glutinosa*. The roots of 2 of the plants were found to contain virus; one produced about 500 lesions on 5 leaves of a plant of *N. glutinosa* and the other about 1000 lesions. The roots of the other 4 plants and of 6 plants tested in a similar manner but not exposed to tobacco-mosaic virus failed to produce infection in leaves of *N. glutinosa* on which they were tested.

Finally, an experiment was conducted as follows: A small glass tube was inserted at an angle into the soil in each of 10 pots in which young Turkish tobacco plants were growing. The tops of the pots were then sealed with paraffin. After 3 days, 10 cc. of a 10^{-1} dilution of tobacco-mosaic virus was introduced through the glass tube into the soil in each of the 10 pots, the end of the tube being subsequently sterilized by heating in a flame. Ten other pots containing Turkish tobacco plants were treated in a similar manner except that no virus was added to the soil. After 2 weeks, during which time none of the plants developed tobacco-mosaic disease, the roots were washed free of soil and tested on *N. glutinosa* for presence of virus. The data from this test are given in table 8. These data and those of the tests mentioned above show that roots of tobacco and *N. glutinosa* plants may be infected with tobacco-mosaic virus merely by addition of virus to the soil in which they are growing. In this respect, therefore, tobacco-mosaic virus behaves in a manner similar to tobacco-necrosis and wheat-mosaic viruses.

CROSS IMMUNITY TESTS.—In symptomatology the virus of tobacco necrosis somewhat resembles viruses of the ring-spot type. Dr. Smith, in a personal letter,

stated that studies on physical properties and host range led him to believe that tobacco ring-spot No. 1, "Bergerac" ring-spot, and tobacco-necrosis viruses are different entities. Further evidence of the distinctive character of tobacco-necrosis virus is afforded by results from recently made cross protection tests. In making these tests, the procedure was to inoculate

crepancy, since he also reported natural infection of plants growing in autoclaved soil watered with boiled tap water. He suggests (Smith, 1937) that these additional cases of transmission are due to the virus being air-borne—i.e., it is carried in the air, falls on soil in which a susceptible plant is growing, and is thence able to infect the roots of this plant. No such

TABLE 8. Numbers of lesions produced in 10 leaves of *Nicotiana glutinosa* with inocula from roots of Turkish tobacco plants exposed to tobacco-mosaic virus and from control roots.

		From plant No.									
		1	2	3	4	5	6	7	8	9	10
Roots exposed to virus	→	0	0	105	71	0	1732	0	0	1038	0
Roots not exposed to virus	→	0	0	0	0	0	0	0	0	0	0

6 young Turkish tobacco plants with the virus to be tested and, after a suitable interval had elapsed, inoculate these same plants with virus of tobacco necrosis. Because of the almost complete localization of the virus, it was not feasible to make reciprocal tests. Plants that had recovered from tobacco ring spot No. 1 and plants recovered from ring spot No. 2 (Price, 1938) developed large numbers of lesions when rubbed with virus of tobacco necrosis. Plants with tobacco mosaic, cucumber mosaic, or severe etch likewise developed lesions when inoculated with tobacco-necrosis virus. Figure 4 illustrates the results obtained in the case of tobacco mosaic. The tests show that tobacco-necrosis virus does not belong in the ring-spot No. 1, ring-spot No. 2, cucumber-mosaic, severe-etch, or tobacco-mosaic virus group.

DISCUSSION.—Viruses belonging to the tobacco-mosaic group have long been considered unique for the reason that they are extremely resistant to various physical and chemical agents. Tobacco-necrosis virus, however, seems to be almost as resistant, in many respects, as the ordinary tobacco-mosaic virus. It has a high thermal death point, is not readily inactivated over a wide range in hydrogen-ion concentration, and is unusually resistant to certain chemical reagents, such as absolute alcohol (Smith and Bald, 1935). It is, therefore, of considerable interest that the virus does not appear to be related to tobacco-mosaic virus or to any other known virus. Because of the distinctive character of tobacco-necrosis virus and its resistance to various chemical and physical agents, it may prove to be useful in further investigations leading to a better understanding of the fundamental nature of viruses.

Smith (1937a, 1937b) reports frequent and rapid spread of tobacco-necrosis virus throughout his green-houses. The writer, on the contrary, has observed no natural spread of the virus in the period of about a year it has been under observation. Not a single case of accidental infection has been recorded during this time. Presence of virus in Smith's water reservoir (1937b) does not completely explain the dis-

sequence of events has been observed in the writer's experience in spite of the fact that there has been abundant opportunity for the virus to get into the air from old infected plants.

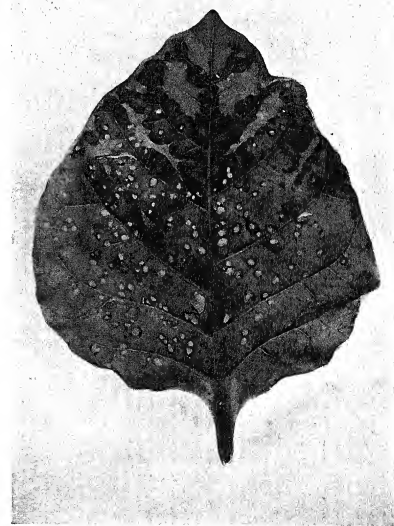


Fig. 4. Leaf of a Turkish tobacco plant showing mottling produced by tobacco mosaic and also necrotic lesions produced by tobacco necrosis. The leaf was infected with mosaic at the time it was inoculated with tobacco-necrosis virus. (Photograph by J. A. Carlile.)

SUMMARY

Symptoms of the tobacco-necrosis virus were found to develop in inoculated leaves of Turkish tobacco within 18 hours. No other plant virus is known to produce symptoms in so short a time.

The virus was found to have a dilution end-point of about 10^{-6} , to have a thermal death point of 90–92°C., and to be relatively stable over a rather wide range in hydrogen-ion concentration. The Black variety of cowpea was found to be a good host plant for use in measuring virus concentration. When the logarithms of the numbers of lesions produced in this host were plotted against the logarithms of the dilutions of virus used for inoculation, the points were found to lie along a straight line with a slope of about 0.7.

No evidence of transmission of the virus by natural means, or of natural occurrence of the virus, was

obtained in the present study. Similarly, it could not be demonstrated that plants are infected by entrance of virus through stomata.

The virus of tobacco necrosis does not ordinarily move from inoculated leaves into other parts of the plant, including the roots. However, K. M. Smith's observation that roots could be infected by pouring virus over the soil around them was confirmed. The tobacco-necrosis virus is not unique in this respect, since it was also found that tobacco-mosaic virus could be transmitted in a similar manner.

Tobacco-necrosis virus was not found to be related to the tobacco-mosaic, tobacco ring-spot No. 1, tobacco ring-spot No. 2, cucumber-mosaic, or severe-etch virus groups.

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A RESPIRATORY STUDY OF POWDERY MILDEW OF WHEAT¹

Paul J. Allen and David R. Goddard

OBLIGATE PARASITES comprise a group of organisms highly specialized from a nutritional point of view. As their name implies, they are restricted to a parasitic existence and cannot be grown on artificial media. This has been an impediment to physiological researches on such parasites and has seriously limited the study of their nutrition and metabolism. In view of the fundamental importance of this aspect of obligate parasitism, a study of the respiration of the powdery mildew of wheat *Erysiphe graminis* D. C. var. *tritici* and of its host was undertaken.

Wheat mildew is an ectoparasite—that is, the only invasion of the host is by haustoria which penetrate the epidermal cells. Thus it is easy to remove all of the mildew, except the haustoria, from infected wheat. This was one reason why *Erysiphe graminis* was chosen for this study.

Trelease and Trelease (1929) studied the effect of the carbohydrate supply of wheat in relation to its infection by mildew. If the carbohydrate supply was cut off by placing plants in the dark, infection by mildew would not occur. Isolated leaves kept in the dark supported the growth of the mildew if their lower ends were placed in solutions of suitable carbohydrates. Development of the mildew under these conditions was independent of the chlorophyll content of the leaves. In the light, growth of the mildew on wheat would occur only if the wheat was supplied with CO₂ or with carbohydrates.

Nicolas (1920) reported that infection of *Prasium majus* by *Erysiphe lamprocarpa* and of *Tonilis nodosa* by *E. communis* brought about a decrease in the respiratory rate of the host. Nicolas did not report on the age of the infection nor the change in the respiratory rate during the course of the disease; therefore it is impossible to decide whether the decrease was the primary result of infection or a change occurring in a late phase of the disease. Yarwood (1934) compared the respiration of clover leaflets infected with *E. polygonum* with that of normal leaflets, and found that the infected leaves had a rate about 50 per cent higher than the normal ones. Since his measurements were of total respiration over a period of several days, they are not directly comparable with those reported in this paper. Pratt (1938) has followed the course of respiration of wheat after inoculation with *E. graminis*. He found that, although the respiration of normal wheat gradually declines with age, wheat inoculated with mildew shows an increasing respiration which gradually rises to a value about three times that of healthy wheat, maintains this level for about a week, and then drops off to zero as the wheat dies. The increased respiration is not appreciably reduced by killing the mildew with sulfur. In a preliminary report Allen and God-

dard (1938) have published some of the results reported below.

MATERIALS AND METHODS.—The first leaves of Marquis wheat plants from 15 to 25 days old, grown in the greenhouse under natural light and at approximately 17°C., were used as experimental material unless otherwise mentioned. Within these age limits, the respiration of the first leaf of normal wheat is fairly constant.

The strain of *Erysiphe graminis* D.C. var. *tritici* used in these experiments was obtained through the courtesy of Dr. Pratt, and was the same as that used by him and by Trelease and Trelease in their studies. Wheat 10 days old was inoculated by heavily dusting the first leaves with conidia. A previous spraying with water from an atomizer insured heavy infection of these leaves, which are particularly susceptible. Infected wheat was kept in a room apart from the healthy wheat, but with the thermostat also set at 17°C. The daytime temperature of these greenhouse rooms actually fluctuated over a rather wide range, but since all of the wheat and mildew in a given experiment was exposed to the same fluctuation, this factor is of minimum importance. Measurements on mildewed wheat and mildewed epidermis were made 7 to 8 days after inoculation, unless otherwise specified.

In the course of this study it was necessary to obtain the epidermis free from the remaining leaf tissues. By carefully scraping a stretched leaf with a razor, all of the green tissues may be removed, so that a strip of epidermis the width of the leaf and a single cell thick is obtained. If mildewed wheat is used, all of the mildew, which invades only the epidermal cells, may be obtained attached to this strip of epidermis. This treatment involves no apparent injury to the mildew.

Measurements of gaseous exchange were made in a Fenn (1928) volumetric micro-respirometer. Since the instrument is closed to the atmosphere at the beginning of an experiment, it is independent of fluctuations in barometric pressure during the course of the experiment. The apparatus used had capillary volumes of about 3.5 c.mm. per cm. of length. The manometers were shaken in a water thermostat at 22° ± 0.005°C. at the rate of 100 times a minute through an arc 2.5 cm. long. Under these conditions the apparatus is sensitive to within ± 0.15 c.mm. per hour. It is a very satisfactory instrument for measuring the small oxygen consumptions reported in this paper. Gas diffusion did not limit the rate of gas exchange in any of these experiments. The respiratory measurements were made in a photographic dark room.

The carbon dioxide produced in respiration was absorbed in KOH cups in the vessels. When working with cyanide, the vapor pressure of HCN was

¹ Received for publication July 7, 1938.

maintained by the use of KCN/KOH mixtures as described by Krebs (1935). Nitrogen used in obtaining anaerobic conditions was freed from traces of oxygen by passing over copper heated to a dull red color in an electric furnace, and was run through the experimental vessels for 5 to 10 minutes. CO was prepared by decomposing formic acid in hot sulfuric acid, washed through a tower of KOH and mixed with the desired proportion of oxygen in a burette bottle. CO/O₂ mixtures and N₂/O₂ mixtures were both made up volumetrically in these bottles.

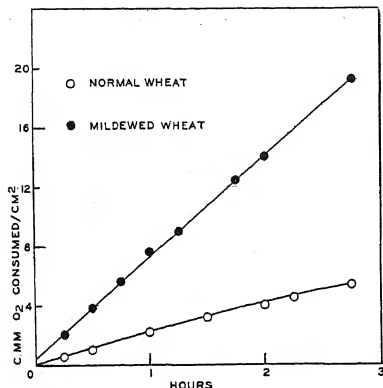


Fig. 1. Oxygen consumption of normal and mildewed wheat. There is a slight decrease in O₂ consumption of normal wheat with time.

As soon as possible before starting an experiment, leaves were cut and placed on moist filter paper in a large Petri dish. For the actual experiments leaves or pieces of detached epidermis were cut into strips about 2 to 3 cm. long; and strips with an area of from 5 to 7 cm.² were floated in each experimental vessel on M/60 phosphate buffer at pH 5.7 to 6.0. Since it was inconvenient to calibrate the amounts of epidermis used except as cm.² of surface, all of the values in this paper are given in terms of gas exchange per unit area (one surface only being considered—not the area of the upper surface of the strip plus that of the lower surface). This permits a direct comparison between a given amount of leaf tissue and the mildew on a similar area of epidermis. In most cases, measurements were also made of the wet weight of the strips of wheat leaves used. The average weight of a normal leaf as determined from 27 different experiments was 16.8 mg. per cm.², with an average deviation from the mean of 1.0 mg. The average of 37 determinations of mildewed leaves was 20.2 mg. per cm.², with an average deviation of 0.96 mg. On the basis of this difference it would, therefore, not be surprising to find values for the oxygen

consumption per cm.² of mildewed wheat which were 20 per cent higher than those for normal wheat. Such a difference is insignificant in comparison with the increase in respiration actually found in mildewed wheat. In obtaining measurements of the respiration of any given type of tissue, two to four separate determinations were made simultaneously in different vessels. In many cases the data presented are representative of a large number of similar experiments.

TABLE 1. Comparison of the respiratory rates of normal and mildewed wheat leaf tissues.

	c.mm. O ₂ consumed / cm. ² surface / hour			
	Determination			Ave.
	1	2	3	
Normal leaf	1.81	1.67	1.68	1.7
Mildewed leaf	10.70	6.60	6.46	7.92
Same leaves, mildew removed	7.40	5.40	—	6.40
Normal epidermis ...	0.15	0.15	—	0.15
Mildewed epidermis .	1.17	1.61	1.21	1.30
Mildewed epidermis, mildew removed ..	0.07	0.20	—	0.14

EXPERIMENTAL RESULTS. — *Respiratory measurements of normal and mildewed wheat and of mildew.*—Wheat which has been infected with *Erysiphe graminis tritici* for 7 to 8 days shows a respiratory rate well above that of healthy wheat of similar age (table 1, fig. 1). In the experiment recorded in figure 1, the increase of oxygen consumption is about 250 per cent. In a later experiment reported in figure 4 the increase was as great as 650 per cent. This increase might be due to the respiration of the attached mildew, or it might represent the combined effect of mildew respiration and an increased host respiration. Since the mildew is an ectoparasite, all of it except the haus-

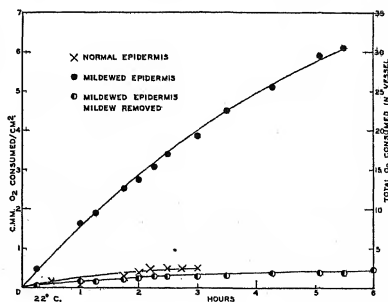


Fig. 2. Oxygen consumption of normal epidermis, mildewed epidermis, and mildewed epidermis after removal of the mildew. The right ordinate shows the actual oxygen consumption in each vessel, the left ordinate the values calculated for a cm.² surface.

toria may be removed from the host with a camel's hair brush without apparently injuring the host. As is shown in the last series of experiments in table 1, this does not reduce the respiration of infected wheat to the level of normal wheat respiration. It therefore seems unlikely that the mildew respiration could account for all of the increased respiration of mildewed wheat.

In order to determine more accurately how much of the respiration of mildewed wheat could be attributed to the mildew, the method described earlier in the paper for isolating mildewed epidermis from the rest of the host tissues was devised, and measurements were made of the respiration of strips of such infected and of normal epidermis. Whereas the respiratory rate of mildewed wheat in the experiment reported in table 1 is 7.9 c.mm./cm.²/hour as against 1.7 c.mm. for healthy wheat, that of mildewed epidermis is only 1.3 c.mm. This is completely inadequate to account for the respiratory increase observed in mildewed wheat. The possibility exists, in fact, that not even 1.3 c.mm. of oxygen is consumed by the mildew on a cm.² of leaf, but that an appreciable part of this oxygen consumption may be attributed to the epidermis. The fact that the respiration of normal epidermis is so low, in one case (table 1, fig. 2) only 11.5 per cent and in another (fig. 4, 7 days after inoculation) 3.4 per cent of that of mildewed epidermis, argues strongly against this assumption. This assumption becomes untenable in view of the fact that upon removal of the mildew from mildewed epidermis the respiratory rate falls to the low rate characteristic of normal epidermis. Since the respi-

ration of normal epidermis is such a small fraction of that of mildewed epidermis, the respiration of mildewed epidermis is an approximate measure of mildew respiration.

From the experiments discussed above, it is apparent that infection with powdery mildew causes a change in the respiration of wheat cells which are not invaded by the parasite nor in immediate contact with it. This suggests that some substance (or substances) produced by the mildew, or whose production in the epidermal cells is stimulated by the mildew, is diffusing out to other tissues and initiating this response. If such a substance is formed, its distribution is not, however, systemic, as the experiments in figure 3 show. In these experiments, several leaves which were heavily mildewed distally, lightly mildewed in their middle portions, and not at all infected basally were cut into three strips, and measurements were made of the respiration of each of the three different portions. Not only is the respiratory increase limited to tissues immediately beneath the infected portions of a given leaf, but there is also a rough proportionality between amount of mildew present and respiratory rate.

Respiratory poisons.—A considerable amount of information has accumulated recently regarding the oxidases which catalyze the reaction between molecular oxygen and substances within the cell. Three distinct oxidases which are known to play a rôle in plant respiration have been found and their properties described. Cytochrome oxidase (phaeohemin or indophenol oxidase) is an iron porphyrin compound whose activity is reversibly inhibited by low concentrations of HCN (Warburg, 1928). In the presence of CO, there is competition with oxygen for the enzyme. The partition coefficient between oxygen and CO is about 9/1 (Warburg, 1926; Keilin, 1930). In a mixture of 90 per cent CO and 10 per cent oxygen, approximately 50 per cent of the enzyme will therefore be in combination with CO, a combination which is enzymatically inactive. In strong light the affinity of CO for the enzyme is greatly reduced and a reversal of CO inhibition is obtained (Warburg, 1928). Cytochrome oxidase occurs in yeast, many bacteria, and many animal tissues, particularly muscle tissue, and has been reported in several higher plants (Kempner, 1936).

A second type of oxidase is the polyphenol oxidase (catechol oxidase) of potatoes and mushrooms, which has recently been shown to contain copper (Keilin and Mann, 1938; Kubowitz, 1937). It is also poisoned by HCN and by CO, but the CO inhibition is not light-reversible. The third oxidase known to occur in plants is the flavine enzyme or "Gelb Ferment" first described by Warburg and Christian (1932a, 1932b; Theorell, 1937). This enzyme does not contain a heavy metal, being a combination between vitamin B₂ phosphate (lacto-flavine phosphate) and a specific protein, and is not poisoned by HCN or by CO. It was first demonstrated in yeast and has subsequently been found in many plant tissues,

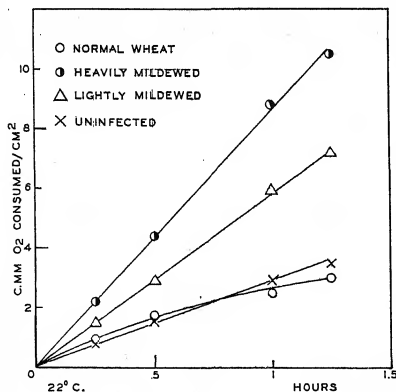


Fig. 3. Oxygen consumption of heavily mildewed, lightly mildewed, and uninfected portions of the same leaves. The curve for normal leaves is included so that a comparison may be made between the uninfected portion of a mildewed leaf and of a leaf from uninfected plants.

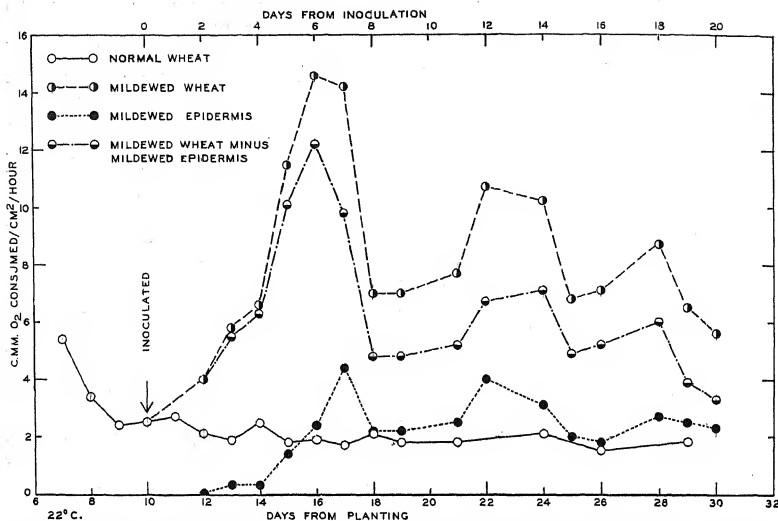


Fig. 4. Respiratory rates as a function of time for normal wheat, mildewed wheat, and mildewed epidermis. A fourth curve is included which is obtained by subtracting the rate for mildewed epidermis from that of mildewed wheat.

notably in germinating seeds of grains, peas, beans, etc. (Euler and Dahl, 1935).

On the basis of these differences in behavior of different oxidases toward HCN and CO, experiments were undertaken to ascertain whether the oxidations in wheat and in mildew were being catalyzed by the same or different oxidases. It was found useful to introduce in this study a third poison, sodium azide (NaN_3), which Keilin (1936) has recently shown inhibits at acid pH the activity of the cytochrome oxidase from yeast.

Healthy wheat respiration is not inhibited by 10^{-3} molar concentrations of HCN, but is rather uniformly stimulated (table 2). This concentration of NaN_3 , however, neither stimulates nor inhibits wheat respiration, nor is respiration inhibited in an atmosphere of 90 or 95 per cent CO (table 2). The slight decrease indicated in table 2 in the presence of these poisons is paralleled by a similar decrease in the control experiments. This slight drop in the respiratory rate of normal wheat during the course of an experiment was a frequent but not invariable occurrence (fig. 1). These data indicate that the oxidase of wheat is neither cytochrome oxidase nor polyphenol oxidase.

As contrasted with wheat, mildew respiration is very sensitive to HCN and NaN_3 , but is unaffected by CO (table 2). In determining the effect of CO on mildew respiration, it was necessary to make com-

parisons simultaneously between different vessels with and without CO, as the rapid decline in respiratory rate (fig. 2) made a comparison over consecutive periods impractical. The failure of CO to inhibit mildew respiration does not necessarily mean that the mildew oxidase is not sensitive to CO. If only part of the mildew oxidase is active in the oxidations going on in the cell—i.e., if the oxidase is present in excess—this extra enzyme could combine with CO without affecting the respiration. The experiments that have been done are not sufficient to distinguish between these two possibilities, but the results with HCN and NaN_3 show clearly that the oxidase functioning in mildew is different from that in wheat.

In view of the different sensitivity of wheat and mildew oxidase toward NaN_3 , it should be possible by differential poisoning to distinguish the respiration of the host from that of the parasite in mildewed wheat. If the conclusion is correct that the increased respiration of mildewed wheat is only in part due to mildew respiration, NaN_3 should eliminate only a part of the increased respiration. The experiments summarized in table 3 show that this is actually the case. When 10^{-3} molar NaN_3 is added to vessels containing mildewed wheat, the respiration decreases, but there still remains the azide-stable fraction which is considerably larger than that of normal wheat. This respiration must be carried over an oxidase similar to that functioning in normal wheat. The reduc-

tion in O_2 uptake brought about by NaN_3 is of the same order of magnitude as the respiration of mildewed epidermis (table 3). The respiration of mildewed epidermis is therefore a reliable measure of mildew respiration. The method of differential poisoning with NaN_3 gives additional confirmation of the conclusion that an increased respiratory rate occurs in the tissues of diseased wheat.

In determining the values plotted in figure 4, eight experimental vessels were used, two containing mildewed epidermis, three each containing normal or mildewed wheat. Each point on a curve is the average of two or three simultaneous determinations. It has been pointed out above that there is frequently a decrease in respiration during the course of an experiment, particularly in the mildewed epidermis, but

TABLE 2. *Respiratory rate in presence of poisons as percentage of control. The figures in parenthesis indicate the number of experiments on which these values are based. The effect of CO was determined in gas mixtures containing 90% CO/10% O_2 and 95% CO/5% O_2 , and compared, respectively, with controls in 90% N_2 /10% O_2 and 95% N_2 5% O_2 . In the case of mildewed epidermis, comparisons are between different vessels during the same time period.*

Tissue	10-3M KCN	10-3M NaN_3	CO	
			90%	95%
Normal wheat	145 (4)	92 (4)	100 (5)	95 (2)
Mildewed epidermis	8 (4)	21 (4)	103 (4)	111 (2)

Changes in wheat and mildew respiration during the course of the disease.—Earlier experiments indicated that there was considerable variation in the respiration of diseased wheat at different periods after infection with mildew. If the course of these changes in respiration were determined, some information might be obtained which would help in interpreting the nature of the changes occurring in wheat under the influence of mildew infection, and perhaps throw some light on the rôle of the wheat in supporting the growth of mildew. A series of experiments was therefore carried out in which measurements were made of the respiration of normal wheat at intervals

also with normal wheat. In order to minimize this source of error, values were calculated for the first hour after equilibrium had been obtained in the experimental vessels. The complete data obtained from the fifth to the eleventh day after inoculating part of the wheat are given in table 4. The close agreement between rates in different vessels lends significance to the variations indicated in figure 4.

The respiratory rate of first leaves of normal wheat is high during the first few days of this experiment, but after nine days from the time of planting, it reaches a fairly constant level which is maintained throughout the remainder of the experiment. On

TABLE 3. *Comparison of the reduction in respiration by azide of mildewed epidermis and mildewed wheat. In this group of experiments no measurements were made of the respiration of normal wheat in azide. In another set, however, the decrease in azide was only 8 per cent.*

Tissue	c.mm. O_2 consumed/cm. ² /hour				Decrease from control value
	1	2	3	Ave.	
Mildewed epidermis	1.61	1.08	1.21	1.30	
Mildewed epidermis 10-3M NaN_3	0.18	0.13	—	0.15	1.15
Normal wheat	1.81	1.68	1.67	1.72	
Mildewed wheat	6.33	5.34	6.46	6.04	
Mildewed wheat 10-3M NaN_3	4.45	3.50	—	4.45	1.39

between 7 and 29 days after planting, and of mildewed wheat and mildewed epidermis at intervals from the time of inoculation until 20 days later. The results are presented in the family of curves in figure 4. In addition, there is included a synthetic curve obtained by subtracting the mildew respiration from the respiration of mildewed wheat and indicating, therefore, the respiratory changes occurring in the host tissues after infection.

inoculation with mildew, however, the rate begins to increase, reaching a value 100 per cent above that of healthy wheat by the end of two days. At this time haustoria have just been formed and the mildew germ tubes are three to four times as long as the spores, but no mildew can be seen with the naked eye, and no measurable respiration of the mildewed epidermis has developed. The wheat leaves still appear dark green and healthy, and do not show outward symp-

toms of disease until four or five days later. The increasing host respiration proceeds for about four days before the mildew has developed far enough to show an independent respiration. During this period and until the maximum is reached six days after inoculation, the increase in host tissue respiration follows a logarithmic curve (fig. 5). Removal of the

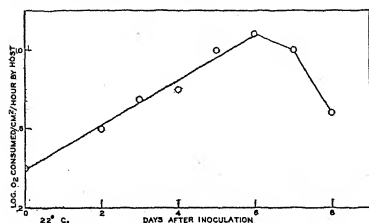


Fig. 5. Log respiratory rate of mildewed host (mildewed wheat respiration minus mildew respiration) as a function of the age of the infection.

mildew from an intact leaf three days after inoculation prevents any further increase in wheat respiration. This logarithmic increase may be interpreted as due to an exponential increase in the amount of substance diffusing into the host from the growing mildew.

The period of logarithmic increase in host respiration will be referred to as the first phase of the infec-

mildew respiration. Whatever this factor may be, its origin is probably in the host, and its continued supply to the mildew controls the respiratory rate there. On interrupting this supply by removing the mildewed epidermis from the host, the respiration of the parasite begins to decrease (fig. 2).

Effect of temperature changes on normal and mildewed wheat.—From a series of experiments in which the respiratory rate of normal and mildewed wheat was measured at five different temperatures between 12°C. and 30°C., it was found that increasing the temperature increased the rate of O₂ uptake in both normal and mildewed wheat. The rate of increase, or the temperature coefficient, is, however, different in the two cases. Calculating the rate of increase as Q_{10} (rate at $t+10$ in degrees Centigrade/rate at t), it appears that normal wheat respiration increases more rapidly over high than over low temperature ranges, whereas the opposite is true of mildewed wheat (fig. 6). The averages of the Q_{10} 's from several different experiments are plotted against temperature in figure 6. Such a marked difference in the change of Q_{10} with temperature in normal and in mildewed wheat indicates some change in the respiratory process of wheat upon infection. This might be interpreted to mean that the reaction limiting the rate of respiration is different in normal and mildewed wheat. Although this interpretation is possible, the data are insufficient to exclude other interpretations, such as a difference in the effect of temperature on the availability of the respiratory enzymes to their substrates.

TABLE 4. Comparison of respiratory rates of different wheat leaf tissues. Each of the figures below represents the O₂ consumed in c.mm./cm.²/hour by the tissue in one experimental vessel. In each vertical column are given the values for one day's experiments, which were used in obtaining points for the curves in figure 4.

Tissue	Days after inoculation						
	5	6	7	8	9	10	11
Normal leaf	2.45	1.81	1.92	2.21	1.30	1.96	—
	2.52	1.80	1.51	1.90	1.50	1.41	—
	1.34	2.16	1.60	—	1.46	2.03	—
Mildewed leaf	11.60	16.70	14.80	6.90	5.86	8.80	11.00
	11.50	14.30	14.45	7.86	8.50	8.10	10.30
	—	12.85	13.60	6.25	6.70	6.27	10.90
Mildewed epidermis	1.86	2.48	4.20	2.30	2.35	2.22	4.32
	1.00	2.33	4.54	2.12	2.07	2.76	3.58

tion, as contrasted with the second phase, the period from the sixth day on. During this second period the host respiration undergoes a series of recessions and progressions, but maintains a constant relation to the mildew respiration (table 5). This relation indicates that there is an inter-dependence of host and parasite respiration. Either the mildew respiration depends on a product of host respiration, or the two are dependent on a common factor whose supply is fluctuating and causing parallel fluctuations in wheat and

Since with increasing temperature the rate at which normal wheat respiration increases is greater than that of mildewed wheat, the difference in respiratory rate between normal and mildewed wheat will be a function of the temperature. It should be expected that at lower temperatures the respiratory increase following infection will be at a maximum.

Fermentation.—The method which has been described for measuring mildew respiration can also be applied in the measurement of anaerobic CO₂ pro-

TABLE 5. Relation between respiration of mildew and of mildewed wheat.

Respiration in c.mm. O_2 / cm.² / hr.

Days after inoculation	2	3	4	5	6	7	8	9	11	12	14	15	16	18	19	20
Mildewed wheat respiration	4.4	5.7	6.6	11.0	14.6	14.3	7.0	6.5	7.4	10.7	10.2	6.8	7.1	8.7	6.6	5.6
Mildewed epidermis ..	0.0	0.3	0.3	1.5	2.4	4.4	2.2	2.0	2.4	4.0	3.1	2.0	1.8	2.6	1.8	2.3
Ratio																
M.W.R. / M.E.R. ...	∞	19.0	22.0	7.3	6.1	3.2	3.2	3.2	3.1	2.8	3.3	3.4	4.0	3.3	3.7	2.4

duction. The experiments shown in table 6 show that the anaerobic CO_2 production of mildewed epidermis, and therefore of mildew itself, is below the experimental error of the apparatus. Any difference in fermentation between normal and mildewed wheat can therefore be ascribed to a change in host fermentation. The data in table 6 show that such a change does occur in wheat after infection. In these experiments the fermentation rate of mildewed wheat is 50 per cent higher than that of normal wheat of the same age. Essentially the same results are obtained whether calculations are made on the basis of wet weight or of area. Not only the respiratory but also

tritici brings about an increase in the respiratory rate of the mesophyll tissue of the host. This increase is roughly proportional to the amount of the infection, and though it occurs in cells not invaded by the parasite, the effect is not a systemic one, but is limited to the tissues immediately underlying infected areas. These results are interpreted as strong evidence of the production by mildew, or, possibly, by the epidermal cells into which haustoria have penetrated, of a diffusible substance which passes out to the mesophyll cells in the immediate neighborhood and brings about an increase in the rate of cellular oxidations involving molecular oxygen. Since the respiratory increase persists after removal of the mildew, it must represent a more or less permanent change in the cells which are affected. Such an increase might result from (a) the production of more oxidase by the host; (b) the production of more of an O_2 carrier, if such a substance is involved in wheat respiration; or (c) an increase in the amount of substrate available for oxidation by the oxidase and oxygen carrier.

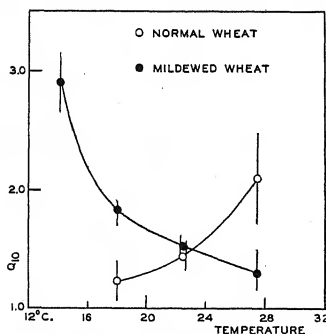


Fig. 6. Temperature coefficients (Q_{10}) of the respiratory rate of normal and mildewed wheat as a function of the temperature. The temperature intervals used were 12-16, 16-20, 20-25, and 25-30°C. The Q_{10} values plotted are the midpoints of these intervals, and the length of the vertical lines give the average deviation of the determinations from the mean. $Q_{10} = (\text{rate at } t_2 / \text{rate at } t_1)^{10/t_2 - t_1}$ where t_2 is the higher temperature.

the fermentation mechanism has been affected by the disease. If the production of CO_2 anaerobically involves steps which also occur in the preparation of substances for oxidation by molecular oxygen, it is possible that a single process in the wheat is being affected by the mildew.

DISCUSSION.—The results presented in this paper show that infection of wheat with *Erysiphe graminis*

TABLE 6. Increase in fermentation of wheat after infection with mildew.

Tissue	Anaerobic CO_2 in c.mm. CO_2 / cm. ² / hr.			Ave.
	1	2	3	
Normal wheat ..	1.76	1.80	1.76	1.77
Mildewed wheat	2.63	2.70	—	2.67
Mildewed epidermis	0.03	0.00	0.08	—

The first possibility seems unlikely for several reasons: (1) If oxidase content were limiting the respiration of normal wheat, an increase in respiratory rate could occur on increasing the oxidase, but not on increasing the available substrate. It has been shown, however, that an increase in respiration may occur when the supply of substrate is increased. In several experiments not reported in this paper, the respiration of normal wheat was increased by adding glucose to the experimental vessels. It has been pointed out above that 1.0×10^{-3} molar HCN also brings about an increase in the respiratory rate of normal wheat. This extra respiration must be carried over oxidase already present in the cell, but

inactive. (2) The frequent decrease in respiratory rate of normal wheat leaves after removal from the plant might be due to a decreasing oxidase content, but it is more probable that the concentration of available oxidizable substrates becomes limiting. (3) If the oxidase were the factor limiting respiration in both normal and infected wheat, the two should show a similar behavior toward temperature changes—which is not the case.

For exactly the same three reasons which have been brought forward in the argument against an increase in oxidase, it seems improbable that the respiratory increase on infection is due to an increase in the amount or availability of an oxygen carrier acting between oxidase and the cell substrates.

The third alternative, an increase in the available oxidizable substrates, might be realized in one of two ways: (1) By an increase in the amount of some enzyme involved in the hydrolysis, dehydrogenation, or activation of carbohydrate or its intermediate breakdown products, or (2) by a change in protoplasmic structure permitting greater activity of some or all of these enzymes. Both of these conditions imply, of course, an increased production of each of the intermediary compounds of carbohydrate breakdown. The effect, then, of the diffusible substance produced by mildew or mildewed epidermis is to increase the amount or activity of one or more of the enzymes involved in the preparation of carbohydrate for oxidation by the oxidase.

It was pointed out above that after the sixth day of infection there is a close parallelism between the respiration of mildew and its host. This relation persists despite a series of major fluctuations. The values for mildew respiration presented in these experiments were taken over the first hour after reaching equilibrium in the experimental vessels, before an appreciable decrease in the respiratory rate could occur. Such a decrease does occur in mildewed epidermis during the course of an experiment, and may represent the depletion of some substance involved in respiration and limiting its rate.

The constant relationship between mildew and host respiration might be explained on the basis of a common factor controlling the respiratory rate in the two. Such a factor could be a substance, either enzyme or substrate, involved in respiration. On removal of the mildew from its host, the supply of this substance is cut off, and that already in the mildew is either used up or lost, with the resulting decrease in the respiration of mildewed epidermis. As long as the mildew remains attached to its host, it will be supplied with this substance in amounts proportional to its concentration in the host.

A morphological examination of the relation between mildew and its host reveals no apparent derangement or injury to the host. No digesting enzymes are produced; no cellular hypertrophy or atrophy occurs. The parasite does not invade the host tissues beyond the epidermal cells, and even in the epidermis produces no patent harm, but lives

symbiotically with the host cells. There is no question, however, that the mildew does eventually cause injury to its host. The pathological condition of infected wheat is due to a physiological derangement, which might be brought about as a result of the abnormally high respiration which characterizes the disease. During the period of maximum respiration six to seven days after infection, the upper limit of photosynthesis is only four or five times the rate of oxidation. This upper limit is probably not reached at all in nature. It is therefore quite possible that the cellular oxidations are proceeding more rapidly than the replenishment of substrate, and that the onset of symptoms, which first appear during the respiratory maximum, is really due to starvation.

SUMMARY

A method has been described for distinguishing between the gaseous metabolism of the obligate ectoparasite *Erysiphe graminis tritici* and that of its host.

The respiration of mildewed wheat is very much higher than that of normal wheat. Part of this extra respiration occurs in the attached mildew, but a larger part occurs in the mesophyll cells of the host, which are not invaded by nor in contact with the hyphae of the parasite.

The respiration of normal wheat is stimulated about 50 per cent by 10^{-3} molar hydrogen cyanide, is unaffected by 10^{-3} molar sodium azide, or by carbon monoxide at partial pressures of 90 and 95 per cent atmospheric.

About 80 to 90 per cent of the respiration of mildewed epidermis is inhibited by 10^{-3} molar hydrogen cyanide or sodium azide.

The respiration of mildewed wheat is reduced in the presence of 10^{-3} molar sodium azide by an amount of the same order of magnitude as the respiration of mildewed epidermis.

The respiration of wheat tissues after infection with mildew rises to a maximum 650 per cent above normal wheat. This increase follows a logarithmic curve, and the maximum is reached six days after inoculation.

Beginning on the sixth day after inoculation the respiration of the host is always about 3.4 times as high as that of the mildew, despite a series of rather large fluctuations in the absolute respiratory rate of both.

The temperature coefficient of normal wheat increases with increasing temperature between 16° and 30°C ., whereas the temperature coefficient of mildewed wheat decreases with increasing temperature between 12.5° and 30°C .

No measurable anaerobic CO_2 production occurs in mildewed epidermis.

The anaerobic CO_2 production of wheat which has been infected with mildew for eight days is 50 per cent higher than that of normal wheat.

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GROWTH OF PHYMATOTRICHUM OMNIVORUM IN SOLUTIONS WITH VARYING AMOUNTS OF CERTAIN MINERAL ELEMENTS¹

C. H. Rogers

In 1934 preliminary field experiments were started wherein varying amounts of certain mineral and other elements were applied to field plots of cotton and cowpeas. The purpose was twofold: First to determine any stimulating effect on host and second any toxic effect on the cotton root rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, one of the limiting factors to cotton and legume production in central Texas. At the same time studies were undertaken in the laboratory to observe the reaction of *P. omnivorum* to these same chemicals in prepared media. Results of laboratory studies are given in this paper.

METHODS AND MATERIALS.—The fungus was grown in 250 cc. Erlenmeyer flasks containing 50 cc. of a liquid medium made up as follows: water, 1000 cc.; ammonium nitrate, 1.2 grams; potassium phosphate (secondary), 1.4 grams; magnesium sulphate, 0.8 gram; potassium chloride, 0.15 gram; sucrose, 30 grams; starch, 30 grams. The media were made to pH 6.8. Reagent quality chemicals and redistilled water were used throughout.

The chemical elements used were copper (cupric sulphate), iron (ferrous sulphate), mercury (mercuric chloride), aluminum (aluminum sulphate), manganese (manganese sulphate), and zinc (zinc sulphate). In-

asmuch as boric acid was used in some field plots, boron was incorporated in the test, using boric acid as the source. To determine any stimulating effect from small quantities of copper, iron, and mercury, these materials were tested in concentrations of from 0.2 to 50 ppm. (parts per million) in three different experiments. In another experiment the other elements were included and the entire group used in concentrations of from 1 to 500 ppm. to establish the maximum tolerance. Stock solutions were made of the different concentrations so that not more than 1.5 cc. were added to each 50 cc. portion of the medium. Flasks were autoclaved after adding the supplementary elements. In the higher concentrations some precipitation occurred regardless of whether the supplements were added before or after sterilization.

The medium was planted with small agar blocks of about 2 mm. dimensions taken from flask cultures of *P. omnivorum* with an approximately 5-day mycelial growth or at the time when the agar medium was just covered with the young whitish mycelium and before the thick buff-colored mats were formed. In one experiment the starch was omitted for the purpose of comparing growth in the starchy and non-starchy media. Cultures were incubated for various periods from 15 to 30 days in the culture room at approximately 25°C. Each test was usually made in quadruplicate and sextuplicate. After harvest, the

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mycelial mats were rinsed with distilled water and dried for 24 hours at 85°C.

RESULTS.—Dry weights of *P. omnivorum* obtained after 17 days in media with copper, iron, and mercury in varying concentrations are given in table 1. Copper began to show some toxicity at concentrations as low as 5 ppm. Growth of *P. omnivorum* in those flasks containing copper at 5, 10, and 25 ppm. was very slow at first but seemed to become more tolerant of the copper as the growth period extended. In

period of 15 days there was a greater growth of the fungus in those cultures containing copper at 1 ppm., iron at 1 ppm., manganese at 1 ppm., and zinc in all concentrations. There was an apparent stimulation in growth from the low concentration of copper and from all concentrations of zinc. In the same manner there was a greater growth in all of the zinc concentrations, except the greatest, during the 30-day period, than the check. In the 30-day period there was an apparent growth stimulus from aluminum at 10 and

TABLE 1. Growth of the root rot fungus during 17 days in culture solutions containing copper, iron, and mercury in concentrations varying from 0.2 to 50 parts per million.

Treatment	Dry weight per 250 cc. flask in milligrams							
	0.2 ppm.	0.5 ppm.	1 ppm.	2 ppm.	5 ppm.	10 ppm.	25 ppm.	50 ppm.
Copper	651	732	708	669	595	502	385	0
Iron	567	635	649	610	631	635	651	590
Mercury	637	651	663	663	609	594	527	568
Check	580							

all flasks containing copper at 0.5 and 1.0 ppm. there was an observable stimulation in growth of the fungus, especially during the first few days of the growth period. This was further evidenced by the increases in dry weights obtained. Initial growth was comparatively slow in cultures containing 50 ppm. of iron and mercury. These too grew more rapidly as the growth period was extended.

Inasmuch as the maximum range of toxicity was not established for iron and mercury, an additional

50 ppm. and from manganese in all concentrations. Zinc caused the most consistent increases in growth, showing some stimulation in all concentrations up to the toxic point of 500 ppm.

In certain cases some rather striking results were obtained. In the higher concentrations of iron, both fungus and medium assumed an iron gray color during the later stages of growth. As the mycelial mats were harvested, they were examined under the microscope to determine what effect the different concen-

TABLE 2. Growth of the root rot fungus in culture solutions containing aluminum, boron, copper, iron, manganese, mercury, and zinc in concentrations of from 1 to 600 parts per million.

Treatment	Dry weight per 250 cc. flask in milligrams									
	During 15 days					During 30 days				
	1 ppm.	10 ppm.	50 ppm.	200 ppm.	500 ppm.	1 ppm.	10 ppm.	50 ppm.	200 ppm.	500 ppm.
Aluminum	174	176	161	0	0	232	274	293	0	0
Boron	204	213	191	0	0	215	110	143	85	0
Copper	233	17	0	0	0	208	254	0	0	0
Iron	209	165	136	136	0	153	201	180	209	0
Manganese ...	214	176	134	0	0	240	284	294	376	319
Mercury	193	186	160	0	0	221	264	362	0	0
Zinc	215	218	227	208	0	248	299	292	236	8
Check	205					230				

experiment was initiated wherein the concentrations were increased to 200 and 500 ppm. Aluminum, boron, manganese, and zinc were included and added in the same concentrations as the copper, iron, and mercury. Average dry weights per 250 cc. flask are given in table 2. Copper again was most toxic, no growth being obtained during either of the two experimental periods of 15 and 30 days at concentrations above 10 ppm. At the end of the experimental

trations had on structure of individual hyphae and mycelial strands and to determine whether there might be some stimulus for spore formation. No spores were found in any of the treatments. In the highest concentrations of boron at which the fungus grew—that is, at 200 ppm.—the cells of individual hyphae were distinctly distorted. The cells were large, usually barrel-shaped, with cell contents of a granular nature. At manganese concentrations of 200

to 500 ppm. the mycelium spread over the entire surface of the medium from the piece of inoculum and later broke up into a number of colony-like mats. The individual mats at the time of harvest were similar to ordinary mycelial mats that are formed under optimum growth conditions—that is, they were very compact and approximately 6 mm. thick. Toward the end of the 30-day growth period these colonies at 200 ppm. of manganese began reuniting into a single mat. Those at 500 ppm. remained distinct, usually six to ten colonies per flask. The compactness and density of the mycelial mats in these high concentrations of manganese account for the increases in dry weight.

Another experiment was set up to duplicate the first tests and to determine the effect of starchy and non-starchy media. *P. omnivorum* has been found by various workers to prefer a starch-containing growth medium. The results of these tests are given in table 3. *P. omnivorum* started growth much more

reported that the disease was not eradicated by the application of 150 pounds of copper sulphate per year, on 1000 square feet of soil, for two consecutive years at College Station, Texas. Ordinarily there is very little rainfall in the central Texas area during the crop-growing season. This usually dry condition or the variation in rainfall coupled with the rather impervious nature of the black soils causes considerable variation in results obtained from the application of dry materials from year to year. It is evident that copper in the form of cupric sulphate is much more toxic to *P. omnivorum* than mercury in the form of mercuric chloride, or any of the other elements tested in the experiments reported in this paper. In addition to the high toxicity of copper, there seems to have been some stimulation of fungus growth from this element in dilute concentrations, as well as from zinc and manganese. It is quite likely that some of these elements were precipitated more than others, especially after the first two or three

TABLE 3. Dry weights per 250 cc. flask of the cotton root rot fungus grown for 3 weeks in culture solutions, with and without starch, containing copper, iron, and mercury in concentrations varying from 0.2 to 50 parts per million:

Treatment	Dry weight per 250 cc. flask in milligrams							
	0.2 ppm.	0.5 ppm.	1 ppm.	2 ppm.	5 ppm.	10 ppm.	25 ppm.	50 ppm.
With starch								
Copper	419	324	423	428	203	98	0	0
Iron	458	408	462	501	473	460	405	109
Mercury	460	499	452	470	455	430	499	474
Check	452							
Without starch								
Copper	53	19	0	0	0	0	0	0
Iron	55	44	36	43	44	31	33	11
Mercury	52	56	86	37	49	35	0	0
Check	70							

vigorously and continued in this manner in the starchy medium as compared with the non-starchy medium. After 3 weeks no growth was obtained in concentrations of copper at and above 1 ppm. At 5 and 10 ppm. of copper in this last experiment on starchy media the initial growth was very slow, increasing proportionately more as the growth period was extended. There was no observed stimulus from copper, iron, or mercury at the various concentrations during the growing period in these last two experiments. The presence of starch in the medium favors a rapid growth of the fungus resulting in about ten times the amount obtained with the same treatment in a non-starchy medium.

DISCUSSION.—Toxicity of copper to *P. omnivorum* has been reported by Bach (1931) and by one or two nurserymen with nurseries located in sandy soils, as well as by the writer (Rogers, 1935). Bach obtained some control with cupric sulphate applied to grapes and citrus trees in the lower Rio Grande Valley of Texas. Taubenhaus and Ezekiel (1933), however,

weeks of the growth period. Changes in hydrogen-ion concentration also occur. The growth stimulus obtained from zinc suggests that this element might be added as a supplement in culture media for *P. omnivorum*. It was particularly noted that although dense mycelial mats were formed in high concentrations of manganese, resulting in high dry weights, few or no sclerotia were produced. In flask cultures under conditions controlled as nearly as possible there are sometimes rather wide variations from flask to flask with a given treatment. Such differences are primarily due to the irregular formation of mycelial strands (Rogers and Watkins, 1938) and masses of sclerotia on the flask walls and the formation of aggregates of pseudo-sclerotia over the mycelial mat or surface of the growth medium.

SUMMARY

The cotton root rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, was grown in flask culture solutions containing aluminum, boron, copper, iron,

manganese, mercury, and zinc in concentrations of from 0.2 to 500 ppm. and dry weights obtained after growth periods of from 15 to 30 days.

Copper as cupric sulphate is highly toxic to the root rot fungus, no growth being obtained above 25 ppm. Growth was inhibited in a non-starchy medium containing as little as 1 ppm. of copper. During the shorter growing periods there was no growth of *P. omnivorum* above 10 ppm. of this element.

Mercury as mercuric chloride was second to copper in degree of toxicity. The fungus, however, was much more tolerant of mercury than of copper.

Zinc in all concentrations up to and including 200 ppm. gave some stimulation to the growth of the fungus. Iron and copper also stimulated growth at certain concentrations. At the end of 30 days, the longest growing period, cultures containing manganese at all concentrations from 1 ppm. to and including 500 ppm. showed a greater growth than the controls. High concentrations of manganese cause a breaking up of mycelial mats to form colonies of a compact nature.

Approximately ten times as much growth of *P. omnivorum* was obtained in a starchy medium as in a non-starchy medium.

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ABNORMAL MITOSIS IN SEEDLINGS OF SOME GRAMINEAE FOLLOWING SEED TREATMENT¹

J. E. Sass

SEEDS TREATED with fungicides are subject to the possibility of injury to the embryo and seedling by the toxic agent in the fungicide. A characteristic type of injury occurs in the seedlings of Gramineae and some Dicotyledonae following seed treatment with dusts containing ethyl mercury phosphate. The malformations and histological changes resulting from this treatment have been described by the writer (1937). Some preliminary observations of the cytological features indicated extensive formation of giant cells, multinucleate cells, and giant polyploid nuclei.

Aberrations of the mechanism of cell division are known to be induced by a variety of external agencies, such as chemical treatment, heat treatment, and irradiation. The extensive earlier literature in this field has been reviewed by Politzer (1934). Recent studies have emphasized the possible genetical use of induced changes of the chromosome complement, especially changes occurring during meiosis. Sax (1937), Blakeslee and Avery (1937), and Nebel and Ruttle (1938) have summarized the recent work in this field. The present study, conducted largely from the point of view of the pathological morphology associated with seed treatment, was undertaken to ascertain the cytological aberrations in treated graminaceous seedlings.

MATERIALS AND METHODS.—For the study of root tip cells, kernels of corn, *Zea mays* L., were soaked for four hours in a 1:2000 suspension of Ceresan, a commercial dust in which the active principle is ethyl mercury phosphate, drained and germinated in a

moist chamber. After an abundance of lateral rootlets had been produced, the paper of the moist chamber was moistened with the Ceresan suspension. With this treatment, many of the root tips that were in contact with the paper became greatly swollen. These tips were removed and processed for sectioning in paraffin, essentially as described by the writer (1937).

Stem growing points and axillary buds of *Avena strigosa* Schreb. and *Holcus sorghum* L. were obtained from dwarfed seedlings produced by heavily dusted grains.

OBSERVATIONS.—The abnormal, swollen tip of a poisoned root exhibits histological features that differ strikingly from normal tissues. In longitudinal sections of a normal root tip the root cap covers the active meristem, and the sloughing edge of the cap extends some distance back from the tip. The meristematic cells are approximately rectangular and are commonly wider than their axial dimension. The cap of a poisoned root is relatively small, covering only a portion of the obtuse, much swollen meristem. The cells of the root cap are not much larger than in the normal root. The meristematic cells of a poisoned root tip are more or less isodiametric, with rounded corners. Some cells approach spherical proportions. Cell size ranges from normal to many times normal size.

The nuclei in the abnormal cells vary greatly in shape and size. The smaller nuclei are usually spherical and may be as small as 15 microns in diameter. Large spherical nuclei attain a diameter of 60 microns. The larger nuclei are more commonly elliptical and

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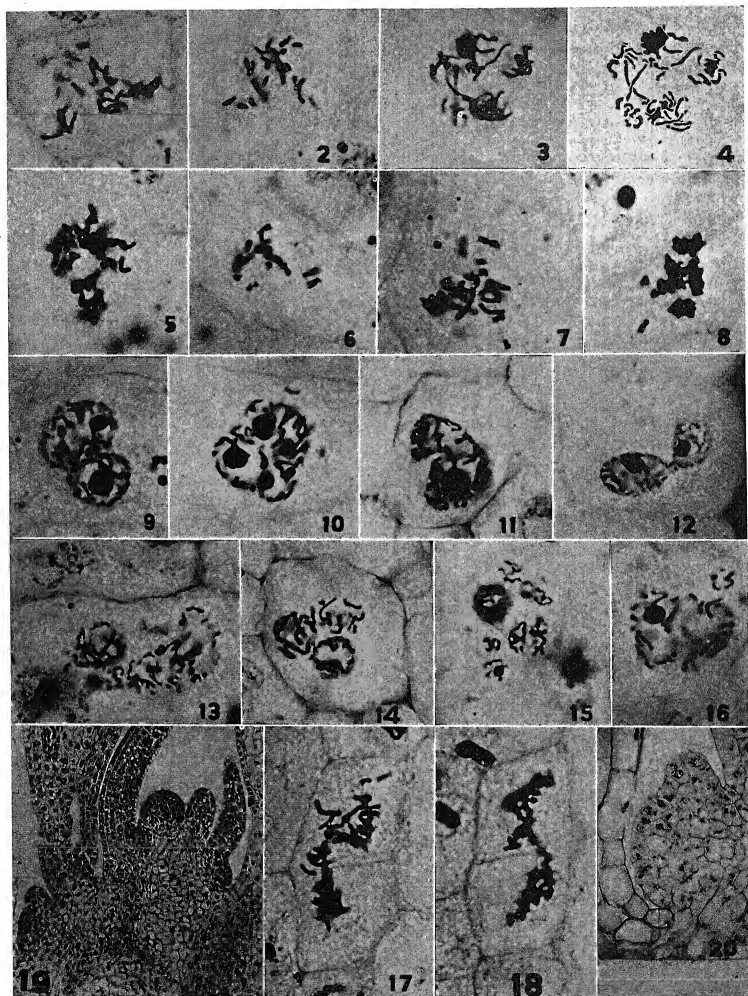


Fig. 1-20.—Fig. 1-18. Root tip cells of corn. $\times 1040$.—Fig. 1, 2. Early anaphase, with poorly defined polarity.—Fig. 3. Late anaphase with five polar groups.—Fig. 4. Drawing of cell in figure 3, showing chromosomes stretched between polar groups.—Fig. 5. Three polar groups evident.—Fig. 6, 7. Separation of entire, split chromosomes from main group.—Fig. 8. Clumping of chromosomes into several groups.—Fig. 9-12. Various degrees of lobing of single nucleus in a cell.—Fig. 13-16. Multinucleate cells, exhibiting various sizes of nuclei in a given cell and in different cells.—Fig. 17, 18. Partial cell plate between chromosome groups in cells having some degree of bipolarity.—Fig. 19. *Avena strigosa*. Growing point, leaf, and axillary bud primordia of seedling just emerging from kernel. $\times 96$.—Fig. 20. Axillary bud in seedling shown in figure 19. Bud has polyploid, multinucleate cells. $\times 184$.

exhibit gradations of lobing, varying from slightly furrowed (fig. 10) to deeply cleft (fig. 11) and approaching nearly complete separation into two or more nuclei (fig. 12). The deeply lobed condition merges into the multinucleate condition (fig. 14).

The origin of the abnormal histological and cytological features described above can be reconstructed from the abundant mitotic stages observable in preparations of poisoned root tips. In any given cell, all the chromosomal material is in the same stage of mitosis, regardless of the size, number, or shapes of the nuclei in that cell.

Early prophase chromosomes appear to have normal structure. The slender, granular threads undergo progressive condensation through the prophases (fig. 13, 16) and presently exhibit the characteristic doubleness of the split late prophase chromosome. In late prophase chromosomes the region of spindle fiber attachment can be recognized. It is possible that a specialist in the morphology of the chromosome complement of corn could identify members of the complement in this pathological material.

The number of chromosomes at metaphase varies within wide limits. Small nuclei in late prophase seem to have less than five chromosomes (fig. 15, 16). At the other extreme are large masses of chromosomes, in which one may hazard an estimate of 200 chromosomes. Between these extremes all gradations of number may be found. The largest numbers were found in apical meristems of the stems of corn seedlings (Sass, 1937).

Abnormalities of chromosome behavior become evident in the anaphase. Normal unwinding and initial separation of the halves of the longitudinally split chromosomes take place, although in some of the chromosomes in this "anaphase" the characteristic doubleness of metaphase persists until the chromosomes are far removed from metaphase position (fig. 6). Poleward movement is extremely irregular. Clear-cut bipolarity is uncommon. Abnormal cells may have as many as six "poles" toward which chromosome movement takes place. The number of chromosomes moving to the more or less defined poles is highly variable (fig. 1-8). It is possible to find figures showing two or three chromosomes removed from the main group (fig. 1, 6, 7, 8). Some figures suggest a more nearly equal numerical distribution to poles (fig. 18). Many figures show attenuated chromosomes stretched between polar groups (fig. 3, 4). Although no direct evidence of fragmentation was found, the evident stresses on stretched chromosomes indicate a possible mechanism for fragmentation.

Following these irregular anaphases, a more or less normal telophase reconstruction of nuclei takes place. A nucleolus reappears and enlarges, associated with each of the lobes of large nuclei (fig. 11), with large separate nuclei (fig. 14), and with some very small nuclei (fig. 15, 16). A nuclear membrane is formed around each chromosome group in a cell. A membrane may envelope only a few chromosomes (fig. 15, 16) or a large group (fig. 10, 16). The degree of lobing in a reconstructed nucleus is determined in

part by the extent of spatial separation during the anaphases.

In strongly poisoned materials great enlargement of cells takes place, associated with nuclear division, but there is little increase in the number of cells. In less severely poisoned material, the cells exhibit a tendency toward bipolarity, there is a more or less precise equatorial separation of chromosomes, and cell plate formation between daughter nuclei occurs in varying degree. The partition may become complete, developing centrifugally in a normal manner and delimiting two daughter cells. The most common aberration is the formation of an incomplete plate, joining a side wall and projecting into the cell (fig. 5, 17, 18). The sharp cleft in the nucleus in figure 11 may be the result of a temporary partial plate of the type shown in figures 17 and 18. The formation of giant cells, either multinucleate or with giant nuclei, is thus the result of aberrant polarity during mitosis and aberrant anaphase separation, usually associated with the failure of cell plate formation.

The foregoing description is based on root tip cells of corn, but similar abnormal cell division was found to occur in all meristematic regions of poisoned seedlings emerging from the kernels of corn, sorghum, and oats. Particular attention is directed to the effect of this chemical stimulation on axillary buds. It was found that the well-defined axillary buds in the germinating embryo of *Avena strigosa* have multinucleate cells and polyploid nuclei (fig. 19-20). Because of the difficulties encountered in the control of dosage, none of the obviously abnormal plants of *Avena* survived beyond the small seedling stage. Consequently, there is no evidence as yet that polyploid cells in the axillary primordia can contribute to the formation of inflorescences.

DISCUSSION.—In the material investigated the apparently normal structure of somatic prophase chromosomes indicates that during the prophases the nucleus is comparatively inert to the poison. Anaphase separation appears to be the stage most subject to drastic disturbance. The present material affords no direct evidence concerning the physical basis for the failure of chromosome separation. Frequent occurrence of one or a few chromosomes well separated from the principal group would weigh against the possibility that pronounced changes of cytoplasmic viscosity interfere with chromosome separation. The multipolar, irregular separation of chromosomes, or the total absence of separation, may be the result of the failure of the spindle fiber mechanism. It would be of interest to test the effect of ethyl mercury phosphate on mitosis in animal cells having a distinctive bipolar centrosomal mechanism associated with the spindle.

The abnormality described here bears striking similarities to the nuclear behavior in cancer cells, as is evident from comparison with Levine's (1931) figures. If this organic-mercury compound is absorbed as readily by animal tissues as by plants, the compound may have some experimental value as a carcinogenetic agent.

In a previous account, dealing primarily with the histological aspects of this hypertrophy, the writer (1936) ventured the suggestion that ethyl mercury phosphate may be of some value in plant breeding work for the production of polyploidy. Subsequent study has shown that the cytological behavior is highly irregular and the dosage is difficult to control, especially with the dormant gramineous caryopsis. The results compare unfavorably with the apparent precision of chromosome doubling obtainable with colchicine. Experiments are in progress to test the effect of localized application of ethyl mercury phosphate on regenerating tissues of some vegetatively propagated plants.

SUMMARY

Ethyl mercury phosphate induces hypertrophy and abnormal mitosis in meristematic cells of seedlings of corn and several small grains.

The toxic agent is absorbed by sprouting kernels and by the root tips of seedlings. The abnormality is produced in axillary bud primordia and other meristematic cells remote from the region of absorption.

Nuclear division is characterized by the formation of multipolar spindles, irregular or incomplete anaphase separation of split chromosomes, and by the failure of plate formation.

Irregular multipolar separation produces multinucleate cells. The nuclei of a given cell may be

approximately equal in size and chromosome number or they may be very unequal.

Total failure of anaphase separation produces giant, polyploid restitution nuclei.

Cell plate formation may be initiated, but such plates are apparently evanescent.

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DIFFERENTIAL GROWTH IN PLANT TISSUES¹

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It was first made clear by Sachs (1882) that in growing plant tissues, the different layers of cells have tendencies to grow at different rates. Both Sachs and Hofmeister (1859, 1860, 1862), as well as still earlier workers, showed that when growing tissues, such as stems and petioles, are cut longitudinally or transversely, the epidermis and generally also the cortex and wood tend to contract, while the pith expands. In the intact tissue, therefore, the epidermis (and in some cases the cortex also) is under tension and the pith under compression. In the case of transverse cuts, the shortening of the external and lengthening of the internal layers is of the order of 5 per cent. These tensions and compressions are not necessarily due to morphological differentiation, since Hofmeister showed that the stalks of mushrooms, which consist of homogeneous hyphae, show a similar phenomenon (see also Discussion). Even single cell-walls cut from long cells of *Nitella* were shown to exhibit tensions. Sachs recognized that "tissue tension is called into being by differences in growth, especially differences in the lengthening of various layers of tissue." The tensions are easily seen by slitting almost any longitudinally growing organ, when the two halves will, in general, curl outward—i.e., the

epidermis side becomes concave; in this way the compression of the pith and the tension of the outer layers are simultaneously released. If placed in water, the tissues continue to grow at different rates—at least for a time—and the resulting outward curvature is therefore intensified.

An important phenomenon of differential growth was discovered in 1934 by F. W. Went. Working on the technique of testing for root formation, Went found that the entry of the root-forming hormone into the apex of the *Pisum* cuttings used in the test was promoted by slitting the cuttings longitudinally. In solutions active in causing root formation, the two halves of the slit cutting curved inward. Since the root-forming hormone was afterwards identified with auxin, the inward curvature became adopted as a simple test for auxin. The extent of inward curvature (see Went and Thimann, 1937) is approximately proportional to the logarithm of the concentration of auxin in the solution in which the slit stems are immersed.

The explanation of this test is in part the subject of this paper. Went's first theory (1934) was that the inward curvature is due to active shortening of the inner tissues, but this was disproved by Jost and Reiss (1936) who studied the comparable phenomenon

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in *Taraxacum* flower stalks and found on measuring the length of the inner and outer sides of the slit stalks that both elongated, but the outer side more than the inner. Van Overbeek and Went (1937), whose data for *Pisum* confirmed those of Jost and Reiss for *Taraxacum*, put forward another theory. According to this, the curvature is due to "the fact that auxin is unable to enter the stem through the wounded surface," but enters wholly, or at least preferentially, through the intact epidermis. Hence this outer layer is the only one to receive appreciable amounts of auxin, and consequently it grows more than the inner layer. This view will be referred to as that of "differential auxin entry."

MATERIALS AND METHODS.—The experiments were carried out with etiolated *Pisum* stems (Alaska peas) and *Avena* coleoptiles (Segerhavre or Victory oats). The plants were grown and all experiments carried out in the dark room with occasional red light at 24°C. and 85 per cent relative humidity. For the inward curvatures, the still growing third internode of the *Pisum* stems was slit centrally for a distance of 3 cm. by means of a special slitter (see van Overbeek and Went, 1937). The tool was fitted with a stop for convenience in getting a uniform length of slit. *Avena* coleoptiles were decapitated 4–6 mm. below the tip and at once slit, free-hand, for 1.5 cm. in the plane of the shorter diameter. Straight growth

TABLE 1. Percentage elongation of 3 mm. sections of peeled *Pisum* stems. Measured at 21 hours after beginning of experiment; 15 sections (from 5 plants) per treatment. Feb. 20, 1938.

Mg./l. indole-3-acetic acid	0	0.001	0.01	0.1	1	10
Elongation when solutions were made up with:						
Distilled water	25	32	36	41	44	42
M/1000 KCl	25	31	43	47	48	49

The idea that auxin cannot readily penetrate through a wounded surface is surprising for several reasons. Firstly, removal of the epidermis is commonly practised to promote auxin entry in a variety of procedures. The adoption of slitting in testing for root formation (see above) is a variant of this. Secondly, in the now standard *Avena* curvature test, the auxin in agar is applied to the transverse cut surface only, yet it enters with great readiness because the test is rapid and extremely sensitive. Thirdly, one of the first demonstrations of the rôle of hormones in growth was that of Cholodny (1926) who introduced coleoptile tips into the interior of bored-out *Lupinus* hypocotyls, the cut inner surface being thus the only path of entry; nevertheless the tips produced a marked acceleration of growth. Lastly, it has since been found that slit *Avena* coleoptiles show the test in the same way as *Pisum* stems, although the coleoptile is a hollow organ with an inner epidermis, and when slit most of the inner side is still unwounded. The sensitivity of slit coleoptiles would then be expected to be much less than that of slit stems, while it is actually about equal to that given with *Pisum* (see fig. 3).

The phenomenon has therefore been reinvestigated from two points of view: (1) To confirm or disprove the theory of "differential auxin entry." This is important from a purely practical viewpoint, because, if correct, all techniques in which auxin is introduced at a cut or wounded surface ought to be abandoned. (2) To gain a better understanding of differential growth. Since normal growth is controlled by the same factors—including auxin—as differential growth, information concerning the latter will be of use in interpreting normal growth.

was measured on unslit 3 mm. sections by means of a binocular dissecting microscope with an eye-piece scale (total magnification 16X). Most of the elongation of such sections occurs during the first two to six hours, but our measurements were generally made after longer periods (usually 36 hours) in order to include as much as possible of the growth. The methods of determining the angle of curvature of slit sections and of removing the epidermis from stems and coleoptiles are discussed in the text. The *Avena* tests were carried out in the usual way. Two decapitations three to four hours apart were made, and curvatures photographed after ninety minutes. All auxin solutions were made up with distilled water.

THE ENTRY OF AUXIN THROUGH CUT SURFACES.—
1. *Elongation of peeled sections.*—If auxin were unable to enter through damaged tissues, the growth of stems from which the epidermis has been removed should not be increased by auxin. Van Overbeek and Went (1937) offer data on this point which indicate that the growth of such peeled stem sections is not increased by auxin. They therefore concluded that auxin did not enter the peeled sections. However, our findings do not support this view but merely show that peeling somewhat reduces the growth response.

Table 1 shows the results of an experiment with *Pisum*. (The data in table 6 provide additional confirmation.) The stems were peeled by "quartering" the bases and tearing off strips of epidermis from the base toward the tip, then "quartering" the tip and tearing off the remaining shreds of epidermis from the tip toward the base. (This double treatment seemed essential to ensure complete removal of the epidermis from the tender growing zone of *Pisum*

stems.) The resulting peeled segments were placed in distilled water for one to five minutes, the three most apical 3 mm. sections cut off and floated on the test solutions. In each of eight series, auxin has been found to increase the growth of peeled *Pisum* sections. From table 1 it may also be seen that adding M/1000 KCl slightly increases the effect of auxin. This holds also for intact sections, but varies greatly from experiment to experiment.² In one experiment growth was also increased by 1 per cent sugar, but as a rule the presence of sugar (even together with phosphate at concentrations from 0.00001 to 0.1 molar) has not increased the growth of *Pisum* sections. There is thus a marked contrast between *Pisum* and *Avena* in this respect.

The results of a test with peeled *Avena* sections are shown in table 2. The coleoptiles were "quarantined" at the base and the epidermis stripped off

tests (not quoted) confirm that auxin strongly increases growth of peeled *Avena* sections. Table 2 also shows that the addition of KCl and sugar increase the growth of peeled sections in auxin.

It is possible, of course, that in these experiments the auxin enters at the transverse cut surface and is transported throughout the section from there. This would imply that entry may occur through a transverse cut, but not through a longitudinal one. That this is not the reason for the good elongation of peeled sections, however, is shown from the data in sections 5 and 6, which in this respect are really more conclusive than measurements of the elongation of peeled sections can be.

It does not appear from these data that the presence of the primary leaf is preventing the entry of auxin into the peeled sections as suggested by van Overbeek and Went. In the experiment of figure 1,

TABLE 2. Percentage elongation of 3 mm. sections cut from peeled *Avena* coleoptiles that had first been immersed 3 hours in distilled water. Measured after 62 hours; 6 sections (from 2 plants) per treatment except where marked *, where twice that number were used.

Mg./l. indole-3-acetic acid	0	0	0	0.01	0.01	0.01	1	1	1
Per cent sucrose	0	0.01	1	0	0.01	1	0	0.01	1
Elongation when solutions were made up with:									
Distilled water	4*	6	8	6	8	17	7*	10	26
M/10,000 KCl	7	8	7	10	9	14	9	12	27
M/100 KCl	9	7	7	7	8	15	18	31	41*

from base to tip, which with *Avena* can be done very readily. The peeled coleoptiles were immersed in distilled water for three hours to allow them to undergo their rapid initial elongation. This rapid elongation of peeled sections, which amounts to about 10 per cent of their length (cf. fig. 3 of van Overbeek and Went, 1937), is more of the nature of a swelling than a growth, and it was thought desirable to exclude it from the measurements. Finally the coleoptiles were cut into 3 mm. lengths, with the primary leaf remaining inside. For comparison with table 2, sections with epidermis intact ("normal sections") in the same solutions grew: in water 12 per cent, in 1 mg. per liter auxin 21 per cent, in auxin plus M/100 KCl 39 per cent, and in auxin plus KCl plus 1 per cent sucrose 74 per cent. Growth of normal sections in the other solutions of table 2 were proportionate. These peeled and normal sections, however, cannot be compared directly because of the long pretreatment of the peeled sections in water. In spite of this and other disadvantages such as injury, the best peeled sections grew more than half as much as the best normal sections of the same day. Two additional

in which additional comparisons of the growth of peeled and normal sections are given, the leaves were removed. The coleoptiles were then peeled, soaked in distilled water for five minutes, cut into sections and mounted on combs. They were then immediately immersed in test solutions. Here again, the peeled sections grew well, especially in presence of sugar, although the whole curve is evidently lowered by peeling.

The data show that: (1) Peeled sections of both *Pisum* and *Avena* respond very well to auxin. In *Avena* it might be objected that the auxin is able to enter through the inner epidermis, but with *Pisum* no such argument can be made. (2) In auxin solutions growth of peeled *Avena* coleoptile sections, like that of normal sections, is increased either by sugar or by KCl or still more by a combination of both. Growth in auxin of peeled as well as of normal *Pisum* stems can be increased by adding KCl. (3) Growth is in general somewhat less in peeled than in normal sections, presumably due to some secondary effect of the peeling—i.e., injury. This is in agreement with the finding of van Overbeek and Went that mere slitting of *Pisum* stems greatly reduces their subsequent growth. For consideration of this damaging effect, see discussion in section 10.

2. *Curvature of peeled slit sections*.—The view of "differential auxin entry" has as corollary that if slit *Pisum* stems are peeled (de-epidermised), they should not exhibit auxin curvatures, because only wounded

² The authors' previous finding (1938) that the growth of *Avena* coleoptile sections in auxin is increased by neutral salts, especially KCl, does not agree with that of Wuhrmann (1937), who found only inhibiting effects. On one or two occasions we have found no effect of neutral salts, but this is exceptional. The data in this paper extend the salt effect to peeled sections and also to *Pisum* stems, thus confirming its general nature.

surfaces are exposed to the auxin, which cannot therefore enter. It will be seen below, however, that such peeled slit stems give inward curvatures of good magnitude when placed in auxin solution. Further, since slit *Avena* coleoptiles also give inward curvatures in auxin solution, the effect of peeling on these was also studied, for in the peeled coleoptile the inner epidermis is the only intact surface, and hence auxin should produce only outward curvature. The experiments show, nevertheless, that peeled *Avena* sections give inward curvature.

In measuring curvatures of these slit stems and coleoptiles, the choice of a reference point is impor-

tant. If the point of inflection between the inward and outward curvatures is taken as reference point (fig. 2A), then differential growth effects which do not give any inward curvature are not taken into account. Thus changes in the extent of outward curvature are overlooked. These may occasionally be important (see sections 7 and 8). The straight unslit part of the stem or coleoptile therefore forms a better reference standard. With this "stem reference" method (sketched in fig. 2B) the water controls give negative curvatures, whose absolute values depend on the length of the slit. When slit for a distance of 3 cm. and placed in water, normal *Pisum* stems exhibit curvatures ranging from -110° to -290° in different experiments, with an average of about -150° , while peeled stems average about -310° . This method of measurement has disadvantage, however, for it adds positive and negative curvatures indiscriminately, and there are cases where this obscures the results, particularly when inward curvature appears near the tip and increased outward curvature near the base of the slit. In some cases below we have therefore quoted results by both methods of measurement. For simplicity and speed, the measurements were made directly on the plants rather than on photographs, with no loss of accuracy.

Figure 2 gives an example of results on *Pisum* by the two methods. It is to be seen that there is neither a direct conversion factor nor a constant difference between the results from the two methods of measuring. For the most intense inward curvatures the values obtained by the two methods approach one another. A further comparison can be made in figure 11.

By either method of measurement, figure 2 shows that auxin curvatures can be obtained with peeled *Pisum* stems as well as with those having the epidermis intact. The actual inward curvature of the peeled stems is less than that of the normal, but the difference between controls in water and those in optimal auxin is about 300° , which is almost exactly the same as for the normal stems. The experiment has been repeated five times, always with the same results, although the absolute curvatures vary considerably from experiment to experiment.

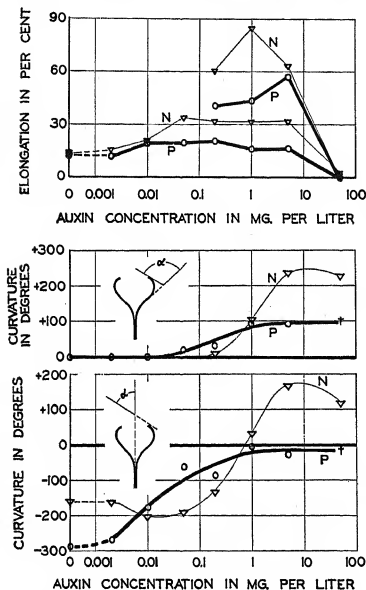


Fig. 1-2.—Fig. 1 (above). Growth of 3 mm. sections of *Avena* coleoptiles in indole-3-acetic acid. Fine line, normal sections; heavy line, peeled sections. The lower pair of curves are for solutions in distilled water; the upper pair of curves for solutions in M/100 KCl plus 1 per cent sucrose. Each point is the average from 30 sections (from 10 plants), except the controls, which are from twice that number. Jan. 26, 1938.—Fig. 2 (below). Curvature of peeled and normal slit stems of *Pisum* when measured by, A, the "inflection reference" method and, B, the "stem reference" method. Sketches in the two figures indicate the two methods of measuring. Heavy line, peeled stems; fine line, normal stems. Daggert indicate that the tissues were dead at the time of measurement. Each point is the average of 12 curvatures, except the controls, which are from twice that number. Feb. 4, 1938.

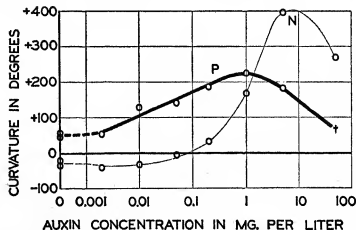


Fig. 3. Curvature of peeled and normal slit *Avena* coleoptiles. Measured by stem reference method. Heavy line, peeled coleoptiles; fine line, normal coleoptiles. Each point is the average of 20 curvatures. Feb. 3, 1938.

Results with peeled and normal *Avena* coleoptiles are shown in figure 3. The effect of peeling is comparable with that in *Pisum* in that the maximum curvature is reached at a lower concentration and is reduced in magnitude.

Combining these data with those of the preceding section we may conclude that: (1) The peeled sections both of *Pisum* stems and of *Avena* coleoptiles give good straight growth, or, if slit, give good differential growth when placed in auxin solutions. (2)

with their bases in a vial of tap water and kept in a moist chamber after application of the paste. Table 3 gives the results of one of our experiments, using a lanoline paste containing indole-acetic acid together with some water, the concentration stated being that in the final aqueous lanoline. Two other experiments gave similar results. Nevertheless, our conclusions are the opposite of those of van Overbeek and Went. The important point is not that the curvature is less when auxin is applied on the inner surface (for of

TABLE 3. Curvature of slit stems of *Pisum* from application of indole-3-acetic acid in lanolin to the cut (inside) surface or to the intact (outside) surface. Measured at about 20 hours; 10 plants per treatment. March 5, 1938.

Method of measurement	Place of application	Control (no lanolin)	Percentage indole-3-acetic acid in lanolin					
			0	0.00001	0.0001	0.001	0.01	0.1
"Stem reference"	Inside	-75, -82	-84	-82	-65	-58	-40	+115
	Outside		-80	-75	-41	+56	+170	+124
"Infection reference"	Inside				0	9	7	146
	Outside			0	9	99	183	160

The peeled sections elongate more in water than the normal (compare also table 6), or, if slit, give larger outward curvatures in water. The explanation of this will be given later in this paper. (3) The lowest auxin concentration causing a detectable increase in curvature is lower for peeled than for normal sections, and correspondingly the lowest concentration which irreversibly damages or kills the sections is lower for peeled than for normal sections. In other

course the auxin gradient will have some effect in opposing the curvature), but that it is in the same direction—i.e., the halves of the stem curve inward.

We may conclude that: (1) The auxin has entered through the longitudinal cut surface. (2) The curvature is not primarily a function of the path of entry of the auxin, but the outer tissue must have grown more than the inner in spite of its being further from the auxin.

TABLE 4. Curvature of infiltrated and not infiltrated slit stems of *Pisum*. Mean of 10-16 curvatures per treatment.

Method of measurement	Date	Mg./l. indole-3-acetic acid	Not infiltrated	Infiltrated by means of aspirator	Infiltrated by means of Hyvac pump
"Stem reference"	Mar. 4	0	-176	ca. -200	
		1	+49	-4	
	Mar. 9	0	-143	-203	-153
		10	+105	+73	+246
"Infection reference"	Mar. 4	1	134	117	
	Mar. 9	10	175	209	298

words, the sensitivity of peeled material to auxin is certainly no less than that of normal material in the same experiment.

The application of auxin in lanoline.—It was found by van Overbeek and Went (1937) that if auxin in lanoline were applied to the slit inner surface of *Pisum* stems, the curvatures so obtained were smaller than when the paste is applied to the undamaged outer side of the stem. We have confirmed this finding. For convenience one of the slit halves of each stem was cut off. The stems were placed upright

4. *The effect of infiltration.*—In tables 5 and 6 of their paper, van Overbeek and Went show that if the sections are infiltrated with auxin by evacuating and releasing the vacuum, they no longer curve inward in 0.3 mg. auxin per liter. The authors interpret this as supporting their view that normally auxin does not reach the inner layers adjacent to the wounded surface, but does so on infiltration. We have not, however, been able to confirm this finding.

Pisum stems were slit and immersed in auxin solutions in the usual way. The Petri dishes were then

placed in a vacuum desiccator and evacuated by means of an aspirator for 5-10 minutes. The vacuum was then released. Table 4 shows that infiltration has little effect on the inward curvature. Repetition of the experiment using a Hyvac oil-pump gave the same results (also in table 4). Both methods of measurement are included in the table.

Since it is possible that infiltration might accelerate or retard growth of both sides, leaving the difference

One reason for the apparent difference between our results and those of van Overbeek and Went lies in the variability of the response of plants on different days. Thus in their figure 5, 0.13 and 0.26 mg. per liter indole-acetic acid at pH 7 gave good curvatures, approximately 30° and 80°, respectively, while in their figure 6 the lowest concentration giving any curvature at pH 7 is about 0.6 mg. per liter (ca. 3×10^{-6} molar). We have found variations of similar

TABLE 5. Percentage elongation of 3 mm. *Pisum* sections in indole-3-acetic acid solutions when infiltrated and when not infiltrated. Measured at 28 hours; 15 sections (from 5 plants) per treatment. Mar. 9, 1933.

Method of infiltration	Mg./l. indole-3-acetic acid	0	0.02	0.1	0.5	2	10
Aspirator	Not infiltrated	15	32	51	53	55	53
	Infiltrated	20	40	44	54	53	56
Hylvac pump	Not infiltrated	17			51	55	52
	Infiltrated	16			51	50	51

between them (and hence the curvature) about the same, we have determined the effect of infiltration on straight growth. Table 5 shows that over a wide range of auxin concentrations infiltration produces only insignificant effects. If peeling were to inhibit auxin entry, the greatest effect of infiltration should be exerted on peeled sections. We have therefore compared the response of peeled and normal sections to infiltration (table 6). The auxin concentration

magnitude. In view of these variations, the use of normal controls on the same day is essential. The concentration of 0.3 mg. per liter which van Overbeek and Went quote as giving no inward curvature on infiltrated plants is very near to the threshold concentration, and, in the absence of controls, it is not even certain that it would have caused curvature without infiltration. Their infiltration experiment on the straight growth of peeled sections is also with-

TABLE 6. Effect of infiltration on elongation of 3 mm. sections of peeled and normal *Pisum* stems. Values are the average percentage elongation of 9-13 sections (three sections per plant).

Date	Time of measurement	Mg./l. indole-3-acetic acid	Peeled		Effect of infiltration	Normal		Effect of infiltration
			Not infiltrated	Infiltrated		Not infiltrated	Infiltrated	
Feb. 24	40 hours	0	31	28	-3	19	22	+3
		1	45	34	-11	54	60	+6
Feb. 27	19 hours	0	13	21	+8	10	12	+2
		1	43	39	-4	43	39	-4
Mar. 4	5 hours	0	17	17	0	14	12	-2
		1	30	27	-3	42	31	-11
	22 hours	0	21	22	+1	18	15	-3
		1	26	33	-3	53	46	-7

here used was about optimal for growth of peeled sections (1 mg. per liter). It may be noted from table 6 that peeling increases the elongation of controls in water, as mentioned in section 2, while it decreases the growth in auxin. However, infiltration has again only minor effects on growth, and tends rather to decrease than to increase the growth of the peeled sections.

out controls that were not infiltrated. In our experiments we have therefore used auxin concentrations near the optimum for curvature and have always included controls at the same time.

Another factor which may have to be considered is the aeration. In some experiments, at least, the bubbling of oxygen through the solution may increase the inward curvatures whether the sections have been

infiltrated or not. Straight growth, on the other hand, was not appreciably affected by oxygenation in our experiments. There are other possible sources of variation even in a technique as closely controlled as this one. However, the differences between the plants on different days are probably the largest source of variation.

In any event, results from infiltration experiments are fully consistent with the deductions drawn in sections 1 to 3.

5. *Auxin curvature in the Avena test.*—The following two simple experiments confirm, in the clearest manner, that auxin enters more readily through a cut surface than through the intact epidermis.

(1) Agar blocks containing 0.05 mg. indole-acetic acid per liter were applied to standard decapitated *Avena* coleoptiles—in one set, on the cut surface in the ordinary way; in another set, on the intact epidermis just below the cut surface.³ Gelatin was used to secure adhesion. The two tests were done at the same time. Plants of equal size were used, and all were decapitated together. The plants with blocks applied to the cut surface gave a curvature of 20.8°, those with the blocks on the epidermis gave 3.3°. The cut surface thus increased the curvature by 6 times.

ordinary way (see fig. 4A). We thus have (a) lateral entry through epidermis, (b) lateral entry without epidermis, (c) (control) longitudinal entry without epidermis. As figure 4A shows, the results were (a) 4.9°, (b) 18.5°, (c) 15.6°. In another experiment the values were 1.3°, 16.5° and 9.6°, respectively.

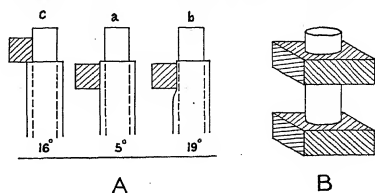


Fig. 4. Explanation in text.

It is evident that, so far from the epidermis promoting entry, auxin enters much more readily in its absence than in its presence.

6. *Direct measurement of auxin movement through wounded surfaces.*—The following experiments give some idea of the readiness with which auxin moves through wounded surfaces.

TABLE 7. *Avena* curvatures from application to test coleoptiles of peeled sections of *Pisum* stems, after immersing in auxin solutions and rinsing in water.

Date	Mg./l. indole-3-acetic acid	0	0.003	0.01	0.03	0.1	0.3	1	3
Feb. 25	Young sections	0.0		7.5	9.9	13.3	18.0		
	Old sections	+0.4				1.8	7.7	13.3	14.2
	Agar block soaked in same solution					14.5			
Mar. 7	Young sections	0.5	0.7	1.8	4.3	7.2			
	Young sections (by diffusion into agar)	0.9		0.9	0.7	3.4	5.2	9.0	
	Agar block soaked in same solution					10.8			

(2) In the above two tests conditions are not quite comparable because auxin entry through the epidermis has to be in a lateral direction, while entry through the cut surface is in a longitudinal direction. Nevertheless, this does not affect the result, as is shown by the following modification. Blocks containing 0.05 mg. indole-acetic acid per liter were applied as before, but two sets were taken for the application to the side just below the cut surface. In one set the epidermis was cut off on this side for just the length of the block (in some plants, a few layers below the epidermis were probably cut off also); in the other set it was left intact. A third set had blocks applied to the transverse cut surface in the

(1) Sections 2-3 mm. long were cut from peeled stems of *Pisum* seedlings, immersed in an auxin solution for a period of about 3 hours, rinsed thoroughly by soaking for 5-6 minutes in distilled water, and finally placed upright on one side of decapitated standard *Avena* coleoptiles. The curvature produced on the *Avena* test plants are shown in table 7. Each figure is the average from twelve sections (three sections from each of four *Pisum* plants) tested on a total of twelve *Avena* coleoptiles. The "young" sections of Feb. 25 are from the tip (second internode) of plants 4 cm. long, and the "old" sections from the same internode of plants 20 cm. long. The higher auxin concentrations needed to obtain curvatures from the "old" sections are probably a result of a greater auxin destruction in this older material. The sections of Mar. 7 are from the growing zone (third internode) of plants 10 cm. long. The values listed

³ Laibach and Kornmann (1933) also obtained curvatures by applying blocks to the intact epidermis, but they did not use decapitated plants and made no comparison between presence and absence of epidermis.

as "by diffusion into agar" are obtained by allowing the auxin from the sections to diffuse into 2.1 per cent agar blocks (1 section per block of ca. 10 mm.³) for one hour and then testing the agar blocks on coleoptiles. In each experiment plain agar blocks were soaked in the same auxin solution to provide a calibration. The experiment has been performed 5 times (with modifications).

When the auxin is first diffused out into agar and the agar tested, it appears that about five times as high an auxin concentration is needed for detection as with the direct contact.

1.5 per cent agar. The whole was encased in a Petri dish in a saturated atmosphere. Transport of auxin took place from the upper block through the side of the stem and out into the lower block, which was afterwards tested on a standard *Avena* coleoptile. Comparison of peeled and normal sections in this experiment is thus a direct comparison between the entry of auxin through the lateral wounded surface and through the intact epidermis. As control against possible diffusion of auxin over wet surfaces, the experiment was carried out in parallel but with the sections inverted. The polarity of a young internode

TABLE 8. Auxin entry through the lateral surface of peeled and normal *Pisum* stems. Method described in text (see fig. 4B). Each value the mean from 12 sections. Positive curvatures indicate curvature toward the block and therefore absence of auxin. May 18, 1938.

Direction of transport	Mg./l. indole-3-acetic acid	0.3	1	3
Normal (apex to base)	Without epidermis	6.1	6.1	9.8
	With epidermis	2.2	5.3	8.2
Inverse (base to apex)	Without epidermis	+1.1	+1.3	+0.3
	With epidermis	+0.6	+0.2	+0.9

The data show that excellent transport of auxin in and out of such sections takes place. The young sections are not much less effective in carrying auxin than are agar blocks. In view of the thorough rinsing to which the sections were subjected, carriage of auxin on the surface can be regarded as practically ruled out.

(2) Six holes, somewhat smaller than the diameter of the uppermost internode of the stem of *Pisum*, were pierced through a 1.5 per cent agar block ($7 \times 10 \times 1.5$ mm.) containing auxin, and through each hole was inserted a section of *Pisum* stem, as in figure 4B. The sections, 4 mm. long, protruded about 1 mm. above the agar, so that their sides, but not their apical cut surfaces, were in contact with it. The base of each section rested on a block of plain

is so marked that any inverse auxin transport would have to be regarded with suspicion. However, no inverse transport took place, so that all the auxin transported must have moved in the normal way through the tissues. To minimize water films also, the peeled sections were not rinsed.

Table 8 shows that the entry and transport of auxin is at least as great in the absence as it is in the presence of epidermis.

Both for this and the preceding experiment, the final curvature in the test coleoptiles was produced by auxin which had passed through no less than three wounded surfaces—namely, entry into *Pisum*, exit from *Pisum*, and entry into *Avena*.

THE NATURE OF DIFFERENTIAL GROWTH.—The above results make it clear that the passage of auxin takes place through wounded surfaces no less readily than through intact surfaces. This is true whether the entry be transverse or longitudinal. Where everything else is comparable, indeed, they indicate that auxin actually enters more readily through a cut than through an intact surface. The inward curvatures of slit pea stems ("pea test") and of other objects in auxin cannot, therefore, be due to differences in the rate of entry of auxin into the wounded and unwounded tissues. Now, if auxin has equal access to all tissues, and curvature nevertheless results, only one conclusion is possible—namely, that the response of the different tissues to applied auxin is different. This conclusion is of considerable importance for the understanding of growth in general. It may provide a physiological basis for the process of differentiation in plants.

It was pointed out earlier in a study of bud inhibition (Thimann, 1937) that the action of auxins on

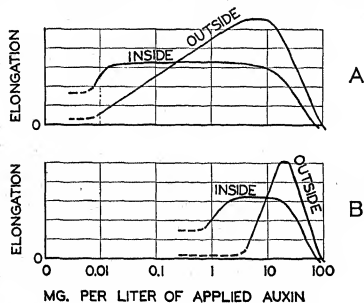


Fig. 5. Schematic curve of the response to auxin by inner and outer tissues of slit *Pisum* stems. A, indole-3-acetic acid; B, benzofurane-3-acetic acid.

the growth of the different organs of a plant, though qualitatively similar, is quantitatively different. The elongation of roots is accelerated by concentrations of 10^{-6} molar and inhibited by 10^{-7} molar or higher, while the elongation of stems is accelerated by concentrations up to about 10^{-4} molar and inhibited by higher ones. The curve of response against auxin concentration is thus of the same general shape for each organ, though shifted horizontally or vertically. The results of the present paper indicate that the same relations hold for the individual layers of cells within a given organ. In the pea stem, the innermost layers—i.e., the pith—reach their maximum elongation at relatively low auxin concentrations, the outermost, on the other hand, at relatively high. The response of the outer layers increases with increasing concentrations up to the maximum which may be tolerated without injury. Thus the outer layers are capable of a much greater maximum growth than are the inner. These relations are shown diagrammatically in figure 5A, which is deduced from a number of experiments on both curvature and straight growth (cf. also figure 1 in van Overbeek and Went, which, however, is plotted on an arithmetic, not a logarithmic, scale).

If this explanation be correct, the different phenomena of the "pea test," whether in *Pisum*, *Avena*, or any other object, would be as the following paragraphs show:

(1) On slitting the stems, the inner layers expand, as a result of the tissue tension. On placing in water, the same phenomenon occurs, since increase of turgor will accentuate rather than release the tensions. On completion of the outward curvature, however, the tensions are still not relieved. This is shown by peeling the epidermis, which procedure leads to a still greater curvature (section 2). It follows that the epidermis must have been opposing the outward curvature to some extent. Since the stem is circular in cross-section, it may be understood that even after it has curved outward, the tension on the epidermis at the sides—i.e., near the cut—has not been released; this will continue to oppose the curvature. This was confirmed by an experiment in which the epidermis was peeled at the sides along the cut only; the outward curvature was then increased even more than by peeling all round. Normal sections gave a curvature in water of -150° , peeled sections -260° , and sections peeled only along the sides of the slit -380° .

(2) On placing slit stems in auxin solutions of moderate concentration, the outer layers extend more than the inner, causing inward curvature. Although the epidermis is not very extensible in water, it must become extensible in auxin (see below) and hence not only does not oppose the inward curvature, but actually plays a part in causing it.

(3) What happens if the slit stem be placed in an extremely dilute auxin solution? It would be expected from the above that here the inner layers should be near their optimum response, while the growth of the outer layers should be relatively little affected. The

absolute amount of growth of each layer might of course still be the same, but it is more probable that the growth of the inside will be actually greater than that of the outside. Curvature in the "pea test" is given by the difference between these two curves, and hence in very low auxin concentrations the slit halves of the stem should curve outward. As will be seen in sections 7 and 8, this expectation is realized for several different auxins. Similar phenomena may be observed with *Avena* coleoptiles and *Taraxacum* flower stalks. The relationship between the two curves is made clear, for indole-acetic acid, in figure 5A, in which, at about 0.01 mg. per liter, the growth of both sides is increased, but the slope of the "inside" curve is greater than that of the "outside"

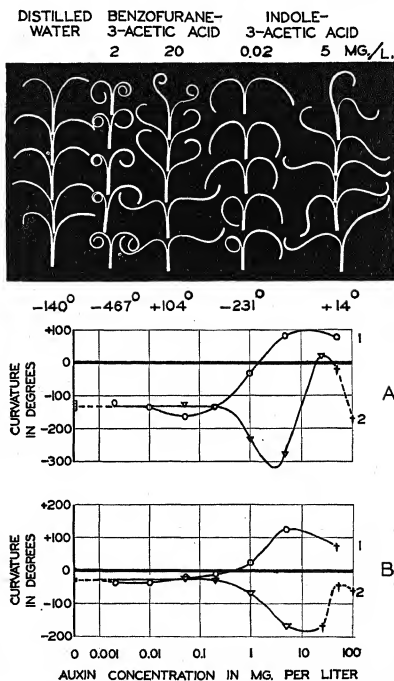


Fig. 6-7.—Fig. 6 (above). Shadowgraph of slit *Pisum* stems showing examples of outward and inward curvatures at about 36 hours.—Fig. 7 (below). Curvatures in indole-3-acetic acid (curve 1) and in benzofurane-3-acetic acid (curve 2). A, slit *Pisum* stems; B, slit *Avena* coleoptiles. Each point is the mean of 16 curvatures. For controls six sets (6×16 curvatures) were used; the values fall within the zero bands plotted. Feb. 21, 1938.

TABLE 9. *Slit Pisum stem curvatures in benzofurane-3-acetic acid and its sodium salt. Each value the mean of 12 half stems, except the water control, which is the average of 60 half stems. Mar. 28, 1938.*

Benzofurane-3-acetic acid, mg./l.	0	0.04	0.2	1	5	25
Free acid ...	—180	—200	—280	—370	+40	
Neutralised ..	—180	—130	—220	—400	+60	

curve. These, and other experiments described below, provide supporting evidence for this conception of differential growth.

7. *The action of benzofurane-3-acetic acid.*—The low but definite activity of benzofurane-2-acetic acid (cumaryl-acetic acid) has been previously described (Thimann, 1935). Because of the interesting properties of this compound, Prof. T. Reichstein of Zürich was kind enough to synthesize its isomer, benzofurane-3-acetic acid (Titoff, Müller, and Reichstein, 1937). This substance is the oxygen analogue of indole-3-acetic acid.

Two explanations are possible for this change of sign. One is that low concentrations of benzofurane-3-acetic acid actually inhibit growth, while high concentrations promote it. This is disproved by straight growth measurements. Sections of pea stems were placed in serial dilutions of the substance and their straight growth measured. Table 10 shows that those concentrations which on slit stems cause large outward curvatures—i.e., between 0.5 and 10 mg. per liter—definitely increase growth. A second experiment gave similar results. It can be seen also from table 10 that neutralization does not change the response.

Since *Avena* coleoptiles also curve outwards in solutions of this substance, straight growth measurements on *Avena* sections are also included in table 10. Again it is clear that low concentrations do not decrease growth. A second experiment gave similar results.

The remaining explanation is, of course, that low concentrations promote the growth of the inner tissues more than that of the outer (see fig. 5B). This is in agreement with all the other evidence and is doubtless the correct explanation.

8. *The action of low concentrations of indole-acetic acid.*—As mentioned above, the response to other

TABLE 10. *Percentage elongation of 3 mm. sections of Pisum stems in benzofurane-3-acetic acid and its sodium salt, also of Avena coleoptiles in the acid. For Pisum, 15 sections (from 5 plants) per treatment; for Avena, 30 sections (from 10 plants) per treatment. Measured at about 48 hours.*

Object and date	Acid or salt	Mg./l. of benzofurane-3-acetic acid or its salt								Mg./l. indole-3-acetic acid 0.1
		0	0.1	0.2	0.5	0.8	2	10	25	
<i>Pisum</i> Mar. 9, 1938	Free acid	14	18		17		19	44	48	51
	Sodium salt	15	17		18		16	45	48	
<i>Avena</i> Aug. 22, 1937	Free acid	6.6		6.7	8.4	8.8	11.6	6.8		16.9

When slit *Pisum* stems are immersed in solutions of this substance, low concentrations give increased outward curvatures, while high concentrations give normal inward curvatures. Figure 6 gives an example of this behavior. The experiment has been repeated ten times with the greatest outward curvature at 2 mg. per liter. The graph of curvature against concentration is given as the curve numbered 2 of figure 7A for *Pisum* and of 7B for *Avena*. These measurements are, of course, made by the "stem reference" method, in which any value less negative than that of the controls is an inward curvature. The points marked with daggers represent plants dead when measured, but as these fall normally on the curve, the auxin response had doubtless taken place before death. In any event, on *Pisum*, 20 mg. per liter of benzofurane-3-acetic acid gives good inward curvatures without killing.

The increased outward curvature is not due to pH, since experiments with neutralized solutions gave exactly the same effect (table 9).

auxins is of the same type. Inspection of figures 2 and 7A shows that indole-acetic acid of about 0.02 mg. per liter definitely increases the outward curvature of slit *Pisum* stems. An example is photographed in figure 6. Most of the outward curvature is located in the base at this time (36 hours after immersion), but at about 60–90 minutes after immersion (see section 11) the curvature is more evenly distributed along the slit section. In contrast, the outward curvature in benzofurane-acetic acid is evenly distributed along the section at all times. However, in high concentrations of both these substances there is a distinct tendency for the base of the slit section to curve outwards more strongly than in water. This suggests that the difference between sensitivities of different layers is not the same in the apex as in the base.

These outward curvatures are of course not shown if the tangent to the point of inflection is used as reference standard. The outward curvatures in indole-acetic acid are small; they are likely to be variable and occasionally do not occur. Nevertheless,

they have been confirmed 19 times. With benzo-furane-acetic acid they are of course much larger and are always readily obtainable. Outward curvatures have also been obtained with low concentrations of indole-butyric, alpha-naphthalene-acetic, and phenyl-acetic acids, and even with γ -phenyl-butyric acid. (The stems in this last case had been decapitated 6 hours beforehand.) It is to be noted that 0.02 mg. per liter is 10^{-7} molar, or, by definition for strong acids, pH 7, so that the outward curvature cannot be due to pH.

The fact that the direction of curvature reverses as the concentration increases may be observed in another connection. If coleoptiles be peeled on one side only, then on immersing in dilute indole-acetic acid solutions, they curve away from the peeled side (cf. the acid curvatures studied by Bonner, 1934). However, in stronger auxin solutions they curve toward the peeled side. An example is given in figure 8, in which curvatures away from the peeled side are recorded as negative, toward the peeled side positive. A second experiment gave similar results. The effect is qualitatively the same whether the primary leaf be left in or removed, the only difference being apparently that the mechanical resistance of the leaf reduces the curvatures somewhat. This bears out the statement made in section 1 that the presence or absence of the fragment of leaf has little effect on the growth of coleoptile sections.

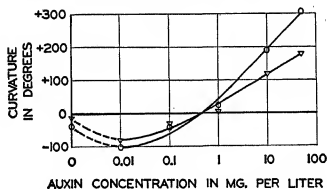


Fig. 8. Curvatures of *Avena* coleoptiles in indole-3-acetic acid when the epidermis has been peeled off one side of the coleoptile. Negative curvatures, away from peeled side; positive, toward. Circles, coleoptiles with primary leaf removed; triangles, coleoptiles with primary leaf left inside. Each point the average from 6 coleoptiles. Measured after about 24 hours. Oct. 15, 1937.

The reversal of sign is not readily explained by simple differences between the rates of entry of auxin into the two sides. It might be suggested that it is due to toxic action of high auxin concentrations on the peeled tissue, since it was shown in section 2 that peeled material is somewhat more sensitive than unpeeled to such toxic effects. This, however, cannot be the explanation, since a decrease of negative curvature begins at concentrations as low as 0.05 mg. per liter, and the curve crosses over to positive values at only 0.5 mg. per liter, which is far below the toxic level. On the other hand, the reversal is explained readily on the basis of differences in sensitivity. One side comprises inner tissue only (peeled side), while

the other side has both inner and outer. As we have seen, low auxin concentrations accelerate the inner more than the outer tissues, so that inner alone would extend more than inner plus outer. In higher concentrations (above 0.5 mg. per liter) the very large response of the outer tissues comes into play, and hence outer plus inner grows more than inner alone.

9. *The behavior of Taraxacum flower-stalks.*—In order to be sure that these phenomena are of general occurrence, it was thought desirable to use some other material. Dandelions (*Taraxacum officinale*) with fully opened flowers were therefore collected. Although when freshly cut the flower-stalks give extremely large outward curvatures in water, Jost and Reiss (1936) found that these were greatly reduced if the heads were cut off twenty-four hours before slitting. In the experiment of figure 9, the flowers were collected and placed in water for one day and the heads then cut off; one series was slit immediately and used (triangles), another series was left for a further day and then slit and used (circles). In both cases low auxin concentrations give an increased outward curvature, while higher concentrations give inward curvatures approximately proportional to the logarithm of the concentration. It is also noteworthy that the plants with heads removed only just before use gave larger outward curvatures in water and in dilute auxin than the others. Benzo-furane-3-acetic acid gave good outward curvature in both groups, the maximum being -308° at 0.2 mg. per liter.

An experiment with etiolated hypocotyls of *Lupinus* also gave the same result. Indole-3-acetic acid at 0.01 and 0.1 mg. per liter increased the outward curvatures by some 30° , while higher concentrations produced inward curvature.

10. *The growth of isolated layers of stem tissue.*—It has been shown that removal of the epidermis of coleoptiles or stems increases the elongation in water but decreases the growth acceleration caused by auxin. The inference from this is that the epidermis must grow little in water (so that it acts to retard extension) but very well in auxin (so that it acts to promote extension of the other tissues). It is also important to note that in general it is inferred that

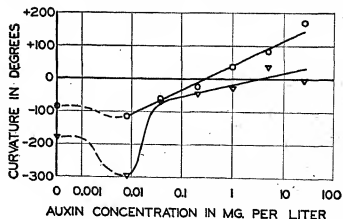


Fig. 9. Curvatures of slit flower stalks of *Taraxacum* in indole-3-acetic acid. Triangles, used immediately after decapitation; circles, used 24 hours later. Each point the average from 14 half stems, except the controls, which are from 28 each. May 3, 1938.

the extension of each cell or cell-layer is influenced by the extension of other cells around it.

In the normally growing plant, with relatively low auxin concentrations, the epidermis will thus tend to lag behind the other tissues, and this agrees with the commonly observed tensions discussed in the introduction. In relatively high auxin concentrations, however, as in the "pea test," which occurs mainly

in auxin concentrations higher than those in the plant, growth of the epidermis should rise above that of the inner tissues.

This is supported by experiments of the following type: Pea stems were placed unslit in optimal auxin (10 mg. per liter indole-acetic acid) for four to six hours and then slit in air. Instead of an immediate outward curvature (as is always obtained when normal plants are slit), most of the stems remained straight or even curved slightly inward. The tissue tension had therefore been almost entirely released by auxin treatment.

As a result of observations on isolated epidermis peeled from *Avena* coleoptiles, Bonner (1934) concluded that the epidermis grows very little in auxin. We have attempted to make similar measurements, but find that the cells of the peeled epidermis are usually coagulated and no growth ensues. In fact they contract from 10 to 15 per cent. Under the microscope it may be seen that the inner wall of such epidermis is largely missing—i.e., the cells have only three sides. Our attempts to obtain epidermis of *Avena* or *Pisum* in satisfactory condition have been fruitless. Intact epidermis has been obtained by peeling *Helianthus* hypocotyls, but here, too, no growth took place in auxin solutions. It follows that the behavior of isolated epidermis cannot be the same as its behavior when in situ. This and other similar phenomena comprise the "damage effect" discussed below.

Measurements, however, may be made on the growth of separated layers of tissue of *Pisum* stems. By making four longitudinal cuts, four slices of outer tissue (with epidermis) and a square central core, about 1 mm. thick, may be isolated. The material is more or less damaged but still grows. Figure 10, which is a mean of four series of experiments, shows the growth of these inner and outer layers in auxin solutions. The inner core grows vigorously in water (probably under the influence of the auxin already contained in it), and its growth is increased significantly by the lowest auxin concentrations used. On the other hand, the outer tissues grow little in water; the slices curve toward the epidermis which thus grows least of all. With increasing auxin concentration up to 10 mg. per liter the growth of the outer tissues steadily increases; in some cases the slices curve away from the epidermis, indicating that the outermost layers have grown most. The lowest concentration which increases growth of the outer tissues is that which already causes maximum growth of the inner layers. To establish this last fact with certainty, the water controls are the mean of 76 sections, and each point on the "inner" and "outer" curves is the mean of at least 38.

If the stems are peeled before cutting ("peeled outer" in fig. 10), the outer tissues are freed from the tension of the epidermis and consequently grow more in water and in low auxin concentrations. However, they are more damaged, as they are now wounded all round. Further, none of these isolated

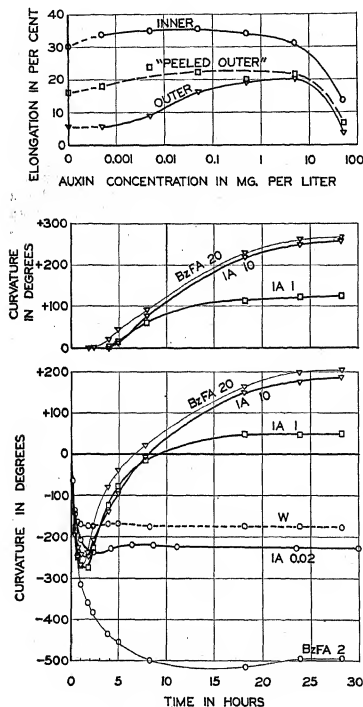


FIG. 10-11.—Fig. 10 (above). Growth of isolated inner and outer layers of *Pisum* stems in indole-3-acetic acid. Discussion in text. The elongations are the averages from 4 similar experiments, except for the "peeled outer" which were included in only 2 of the 4.—Fig. 11 (below). Change of curvature with time after placing in auxin solutions. *Pisum* stems, slit in usual way; A, measured by the "inflection reference" method; B, by the "stem reference" method (cf. fig. 2). Lettering of curves:—W, distilled water; BzFA, benzofurane-3-acetic acid 2 and 20 mg. per liter; IA, indole-3-acetic acid 0.02, 1 and 10 mg. per liter. Each curve the average from 10 half-stems. All of April 9, 1938, except IA 0.02 mg. per liter which is of Mar. 31, 1938.

tissues grows as much as in the intact stem, whose elongation in twenty-four hours often exceeds 50 per cent. Thus the difference between the "outer" curve of figure 10 and that of figure 5A, which is deduced from the slit stem curvatures and from growth of intact sections, represents the effect of the damage. This decreases the response to auxin, particularly to the higher concentrations, and thus makes the quantitative interpretation of these curves somewhat uncertain.

In other experiments the central core was bored out by means of a very thin-walled glass tube, and the growth of the resulting hollow cylinder of outer tissue compared with that of the inner core.⁴ Table 11 shows an experiment in which two diameters of glass tube were used. As above, in no case do the isolated layers grow as much as the intact sections from the same group of plants. When the boring is small, the inner core grows no more in auxin than in water. The coarser boring, however, yields an inner core which shows definite response to auxin. In both cases the outer layers grow less than the inner in water, but very much more in auxin. In other words, had the sections been slit, the growth differences would have led to outward curvature in water and inward in auxin. These data confirm those above in

in table 11 neither the inner nor the outer tissues grow as much as the intact material, this damage effect is evidently a large one. We have considered the possibility that something is being lost from the tissues through the cut surface, but the evidence for this (see also Schneider, 1938) is inconclusive. The effect is not likely to be due to auxin destruction, since it persists in presence of large volumes and reasonably high concentrations of auxin solutions.

Whatever its explanation, we believe that this "damage effect" is in part responsible for the reduced growth of peeled sections in optimal auxin concentrations and for the failure of the "inner cores" of *Pisum* in some experiments to respond to auxin at all. On this account it must also contribute to the inward curvatures in the "pea test."

11. *The change of curvature with time.*—It was pointed out in section 2 that if the unslit stem of *Pisum* is used as reference line for the curvatures, changes in outward curvature are readily determined. We have therefore followed the change of curvature with time, using this method. The results of one of three similar experiments, using those concentrations of indole- and benzofurane-acetic acids which give optimal outward and inward curvatures, are plotted in figure 11. They confirm and extend those of van

TABLE 11. *Percentage elongation of bored-out stems of Pisum and of the removed inner core. Each value the mean of nine 3 mm. sections.*

Solution	Intact	Small boring		Large boring	
		Outer layers	Inner core	Outer layers	Inner core
Water	13.6	7.4	8.6	5.5	11.7
Indole-3-acetic acid 5 mg. per liter ...	35.9	21.2	8.1	21.4	15.0

showing directly that there is a difference in auxin response between the inner and outer tissues. The different behavior of the coarse and fine inner "cores" suggests further that this difference in auxin response grades through the tissues from pith to cortex. Another experiment gave similar results, except that growth of the inner cores in water was greater.

From all these experiments on isolated tissues the average maximum increase over water controls (in percentage of the length) caused by optimal auxin concentrations was: for the outer tissues, 20 per cent, for the peeled outer tissues 9.2 per cent, and for the inner tissues 5.2 per cent.

The reduction in growth resulting from damage calls for further consideration. It is shown very clearly in table 11 and figure 10, as well as in other experiments in this paper. Similarly it was shown by van Overbeek and Went that slitting of pea stems greatly reduces their growth in water or auxin. Since

Overbeek and Went (1937) with the difference that they show clearly that in all cases the initial response is an increase in outward curvature. Measurements of the same stems by the "inflection point" reference method, shown in the upper half of the figure, fail completely to show this.

Only after one to two hours does the tendency to curve inward become apparent. This change in direction is probably due to the more rapid entry of auxin on the cut side, causing a temporary acceleration of the growth of this side, which is then superseded by the increased response of the outer side. This supports previous indications that on the whole auxin enters more rapidly, rather than less rapidly, on the cut side.

DISCUSSION.—In all the experiments reported above no attempt has been made to identify "inner" and "outer" layers with histologically defined tissues. The impossibility of making a longitudinal cut exclusively through any given tissue in a radially symmetrical object has seemed to make this unnecessary. Presumably the inner core in *Pisum* stems consists wholly or mainly of pith parenchyma, but that is all that can be said. Some finer development of the

⁴ The hollow cylinders became very thick in the auxin, reaching perhaps double their original diameter,—i.e., they were growing more in the transverse than in the longitudinal direction. Hence in the intact section this transverse growth, of which the epidermis is evidently capable, is prevented by the action of the inner tissues.

boring technique might be applicable to this problem, but for the present the exact nature of the tissues concerned must be left open.

On account of the "damage effect," other slit tissues and organs have been surveyed for suitability as test objects, but none seems notably better than the material used. *Allium* leaves give extremely large outward curvatures in water; *Pelargonium*, *Oxalis*, and *Tropaeolum* petioles give inward curvatures in auxin, but of only low sensitivity; *Allium* roots give inward curvatures in water alone, which are not changed in sign, but merely reduced or inhibited in auxin according to the concentration used. It is of interest to note that the behavior of roots is thus entirely different from that of shoots. *Agaricus* stalks (cf. Hofmeister, 1860) give inward curvatures in water also, and these, like those of roots, are not altered in sign by auxin of any concentration tried.

In the conception of growth which we have tried to bring out in this paper, certain points call for emphasis.

Growth by elongation is evidently a composite phenomenon. Some cells, if left alone, would grow more than they do in the intact tissue, others less. This is essentially Sachs' view of tissue tensions. Since growth is controlled by auxin, the theory that each cell layer has a different response to auxin provides the mechanism for these different growth tendencies.

As a rule, under normal physiological conditions the auxin concentration in elongating tissues is relatively low, so that since the response of the inner tissues is optimal in low concentrations, these layers have more tendency to grow than the outermost. Thus it is that the inner layers are under compression, the outer under tension.

Bonner (1935) found good evidence that in *Avena* coleoptiles the cellulose micelles in the epidermal walls are oriented parallel to the longitudinal axis, while in the other longitudinal walls they are more nearly perpendicular to this axis. This fact receives ready explanation from the tension which is continually being exerted on the epidermis during growth. Bonner's data show how readily an artificially applied tension causes change of orientation of the micelles into the direction of the tension.

On the other hand, the inward curvatures of the "pea test" are obtained in auxin concentrations higher than those normally present in the plant, although very rapidly growing internodes do occasionally exhibit small inward curvatures at their tips in water alone. (These are presumably due to relatively large auxin concentrations present in this material.) The really large inward curvatures are essentially the result of the shape of the auxin-response curve for the epidermis or perhaps for the layers immediately beneath it. This curve continues upward in high auxin concentrations, while the curve for the inner tissues reaches its maximum at a low concentration and at higher concentrations either remains stationary or decreases somewhat.

The conclusion that the curve of growth response against auxin concentration differs not only for different plants and different organs, but also for different tissues within the same plant, is not a surprising one. We know, for instance, that cambium cells divide when provided with auxin, while other cells do not. Differences in access of oxygen, in rate of water entry, in wall thickness and orientation of wall constituents, and finally in the supply of factors other than auxin, all doubtless play a part in causing the differences. There is another factor which may be of importance—the difference in response may be really a different enlargement in presence of equal internal auxin concentrations, but it may also be due to the establishment of different internal auxin concentrations in the cells. In other words, the tissues may have different ability to accumulate auxin. Some evidence for this is provided by experiments with pyrrole-2-acetic acid, which has very low auxin activity and is rapidly broken down to a black oxidation product. Pea stems immersed in solutions of this substance show very marked blackening in and around the phloem and in the cambium, but only very slight blackening in the pith parenchyma and the cortical cells. This could be due to vigorous accumulation of the substance by the former groups of cells. However, the effect may, of course, be due to differences in enzymatic activity, so that the argument is not conclusive.

The fact that the relative effectiveness of different auxins also varies from plant to plant may involve some or all of the above causes. Thus, even in straight growth of immersed sections, where transport and other secondary factors are as far as possible excluded, there are still large differences in relative activity between *Pisum* and *Avena*. The causes of these differences will doubtless be elucidated by further study.

SUMMARY

It is shown in a variety of ways that the entry of indole-3-acetic acid into plant tissues takes place at least as readily through cut and wounded surfaces as through the intact epidermis. The evidence indicates that the entry is more rapid through wounded surfaces than through intact, but the final state reached is probably the same in each case (sections 1 to 6 and 11).

Slit *Avena* coleoptiles and *Taraxacum* flower-stalks, on immersion in auxin solutions, behave in the same way as *Pisum* stems (sections 2 and 9).

The inward curvature of these slit organs on immersion in auxin cannot be due to differences in the rate of entry of auxin through the intact and wounded surfaces, but must be due mainly to differences in the responsiveness to auxin of the inner and outer layers of tissue.

The behavior of benzofurane-3-acetic acid, which produces increased outward curvatures in all but very high concentrations, supports this view (section 7).

Further support is derived from the fact that other auxins, in concentrations too low to cause inward

curvatures, also increase the outward curvature of such slit organs (section 8).

Direct measurements on the elongation of isolated layers of tissue confirm that the inner and outer layers behave differently in auxin, the inner layers showing most of their auxin response in very low concentrations, the outer layers in very high ones (section 10).

The operation of isolating such tissue layers, or in general any wounding process, introduces a "damage

effect" which decreases the subsequent response to auxin (section 10).

The differences in the auxin response of different tissues are the principal cause of the "tissue tensions" studied by the older botanists.

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THE OCCURRENCE OF CEDRUS IN THE AURIFEROUS GRAVELS OF CALIFORNIA¹

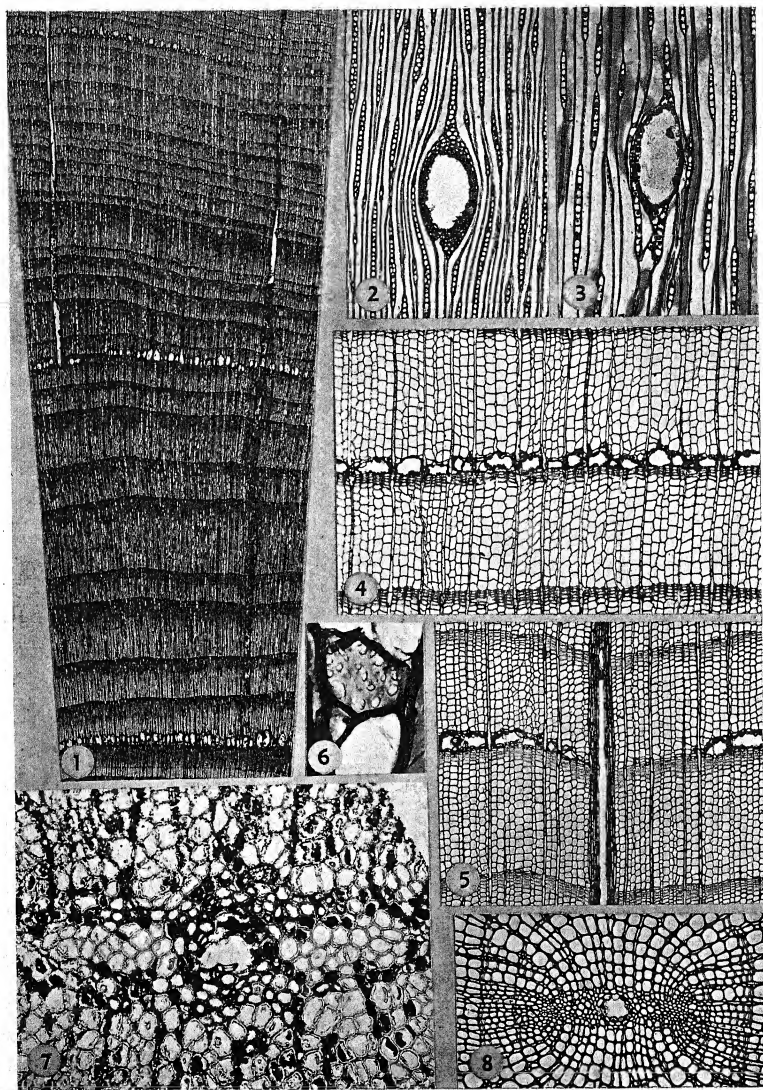
E. S. Barghoorn, Jr., and I. W. Bailey

THE AURIFEROUS gravels of California frequently contain logs and smaller fragments of wood, which, owing to their remarkable state of preservation, have aroused much interest among both laymen and scientists. Although buried many millions of years ago, the specimens are not mineralized, lignitized, or carbonized, but closely resemble woods which have merely become brittle after prolonged immersion in water. When exposed to the air, they are, at times, attacked by insects and fungi, and, when sectioned, may be differentially stained with safranin and Heidenhain's haematoxylin. The walls of their constituent cells still exhibit typical anisotropy in polarized light and have been shown by Mitchell and Ritter (1934) still to contain pentosans, cellulose, and lignin, although in somewhat modified ratios.

¹ Received for publication June 6, 1938.

Many of the specimens from the auriferous gravels obviously came from trees of large size and exhibit superficial resemblances in color and texture to the wood of *Sequoia*, to which genus they are commonly assigned by most individuals who have examined them. A microscopical study of a number of the reddish-brown specimens from Eldorado, Placer, and Yuba counties reveals the fact, however, that at least three genera are represented among these woods—viz., *Sequoia*, *Libocedrus*, and *Cedrus*. The occurrence of *Cedrus* is particularly significant, since this genus has not been recorded previously from the Mesozoic or Tertiary deposits of western North America. It seemed advisable, therefore, to make a detailed investigation of available material, the results of which are recorded in the following pages.

MATERIAL.—Wood of *Cedrus* was obtained from five different localities:



Specimen 1.—Section of what appears to have been a layered stem or branch bearing lateral shoots and roots. Collected by District Ranger R. C. M. Berri-man in an abandoned gold mine known as Roanoke Tunnel near Bottle Hill, Eldorado County. Wood of *Sequoia* was also obtained from this mine. Specimens contributed by L. H. Daugherty and C. L. Hill.

Specimen 2.—Wood from the outer part of the stem of a large tree. Collected by Ranger Berri-man in the Ralston Ridge Mine of the California-Hawaiian Development Company, Eldorado County. Wood of *Libocedrus* also obtained from this mine. Specimens contributed by C. L. Hill.

Specimen 3.—Wood from the outer part of the stem of a large tree. Collected by Ranger Berri-man in the Glen Mine, Placer County. Wood of *Sequoia* also obtained from this mine. Specimens contributed by the U. S. Forest Service.

Specimen 4.—Wood from the outer part of the stem of a large tree. From auriferous gravels underlying lava, Sierra Nevada. Exact location of mine not known. Specimen contributed by Professor E. W. Berry.

Specimen 5.—Wood from the outer part of the stem of a large tree, Placer County. Specimen No. 7354 in the paleobotanical collections of Harvard University, bearing the following inscription: "From tunnel No. 1, Central Pacific R. R., Blue Cañon, Sierra Nevada Mountains. Elevation above sea level 4520 feet. Found under 60 feet of conglomerate." Specimen loaned by W. C. Darrah.

The auriferous gravels of California are commonly assigned to the Miocene period (Lindgren, 1911); but data are accumulating which indicate that the deposits may range in geologic age from Eocene to late Miocene (Chaney, 1932). According to the best available evidence, the woods from the mines of Eldorado and Placer counties are from fluvial sediments of a Miocene drainage.

DIAGNOSTIC CRITERIA IN IDENTIFYING THE WOOD OF CEDRUS.—Bailey and Faull (1934) have shown that the secondary xylem of the Pinaceae,² with the exception of certain species of *Pinus*, may be distinguished from that of the remaining representatives of the Coniferae by fundamental differences in the walls of the parenchymatous cells. In *Picea*, *Larix*, *Pseudotsuga*, *Cedrus*, *Keteleeria*, *Pseudolarix*, *Tsuga*, and *Abies*, the cells have both primary and secondary walls (fig. 10, 12), whereas in the Taxaceae, Cephalotaxaceae, Podocarpaceae, Araucariaceae, Taxodiaceae, and Cupressaceae, the parenchymatous elements are provided with a primary wall only (fig. 9, 13).

² Nomenclature of Pilger (1926).

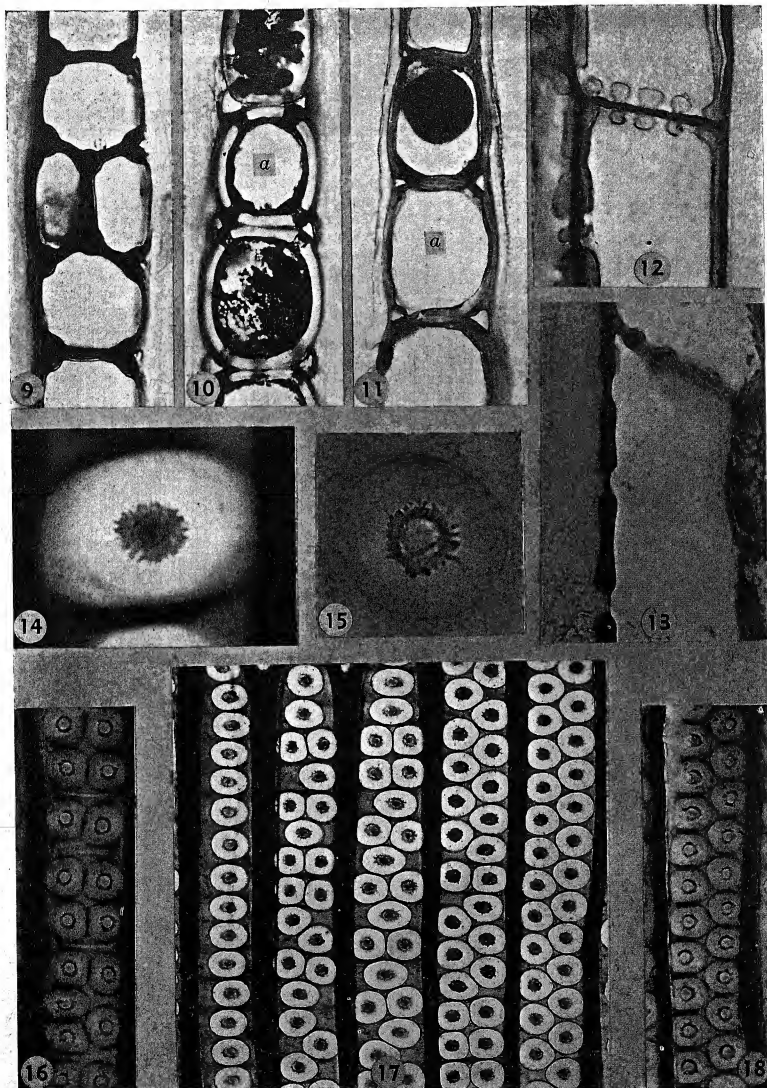
As defined by Kerr and Bailey (1934), the primary wall is formed in growing and dividing cells where it is capable of increase in surface area and of undergoing various reversible changes—e.g., in thickness. Morphologically, it is characterized by being perforated by more or less numerous plasmodesmata which not infrequently are aggregated in thinner parts of the wall—i.e., in so-called primary pit-areas or sieve fields. During tissue differentiation, the primary wall may retain its original thickness, or it may become thicker or thinner. The secondary wall is a supplementary layer formed by cells which have attained their final size and form and are undergoing various irreversible changes. This type of wall has primarily a mechanical function and, once formed, appears to be incapable of growth in surface area. It is characterized by having relatively large perforations—i.e., simple or bordered pits—and is devoid of plasmodesmata.

It should be noted in this connection that deeply depressed primary pit-areas in thick primary walls (fig. 13) may at times bear a superficial resemblance to the pits of secondary walls (fig. 12). Therefore, it is essential, particularly in dealing with the wood parenchyma and ray parenchyma of fossilized coniferous xylem, to have some specific structural feature which may be utilized in detecting the presence of a true secondary wall. In the Taxodiaceae, Cupressaceae, etc., which have primary walls only in such parenchymatous cells, there are no pitlike depressions in those parts of the wall which border upon the intercellular spaces (fig. 9). On the contrary, in the Abietoideae,³ the secondary wall is provided with simple pits, many of which are oriented towards the intercellular spaces (fig. 10); but the pit cavities of these are not confluent with the intercellular spaces, since they are separated from them by intervening parts of the primary wall. Thus, in the case of coniferous woods, the presence of pits directed towards the intercellular spaces is an indication of abietean affinities.

Strasburger (1891) erred in stating that such pits occur in the rays of *Juniperus*. In lignified ray parenchyma of the Taxodiaceae, Taxaceae, Cephalotaxaceae, Podocarpaceae, and Cupressaceae, the parts of the primary wall which border upon the intercellular spaces commonly are unligified or less intensely ligified. Therefore, as seen in tangential longitudinal sections of the xylem, the portions of the wall between the lumen and the intercellular spaces frequently exhibit a different color or index of refraction. That there are no pitlike depressions

³ Nomenclature of Pilger (1926).

Fig. 1-8.—Fig. 1. Specimen No. 1. Transverse section of the xylem, showing two types of "traumatic" resin canals. $\times 11$.—Fig. 2. *Cedrus atlantica*. Tangential longitudinal section of the xylem, showing horizontal resin canal in sectional view. $\times 50$.—Fig. 3. Specimen No. 4. Tangential longitudinal section of the xylem, showing horizontal resin canal in sectional view. $\times 60$.—Fig. 4. Specimen No. 4. Transverse section of the xylem, showing "traumatic" resin canals. $\times 35$.—Fig. 5. *Cedrus atlantica*. Transverse section of the xylem, showing both vertical and horizontal resin canals. $\times 50$.—Fig. 6. Specimen No. 1. Tangential longitudinal section of the xylem, showing simple pits in the end wall of a ray cell. $\times 800$.—Fig. 7. Specimen No. 1. Transverse section of a root, showing centrally located resin canal between the two arms of primary xylem. $\times 110$.—Fig. 8. *Cedrus libanotica*. Transverse section of a root, showing a similar type of structure. $\times 50$.



in these parts of the primary wall may be demonstrated by careful focusing or by examining transverse and radial longitudinal sections.

The ray parenchyma of specimens 1-5 from the auriferous gravels of California have clearly differentiated secondary walls and numerous pits directed towards the intercellular spaces (fig. 11). It is evident, accordingly, that these specimens are not the wood of *Sequoia*, but of some representative of the Pinaceae. This conclusion is strongly supported by the structure of the lateral roots embedded in the Bottle Hill specimen. As shown in figure 7, the diarch roots have a median resin canal located between the two strands of primary xylem, a structural feature that is characteristic of *Cedrus* (fig. 8), *Keteleeria*, *Pseudolarix*, *Tsuga*, and *Abies*, but not of the Taxodiaceae. Furthermore, as noted by Jeffrey (1905), the roots of *Pinus*, *Picea*, *Larix*, and *Pseudotsuga* have a resin canal confronting each arm of the primary xylem—i.e., two in a diarch root, three in a triarch root, etc. Therefore, these genera are excluded from consideration in identifying the Bottle Hill specimen.

Figure 1 illustrates a transverse section of a part of the main axis of the Bottle Hill specimen. At the right and left sides of the figure, radially oriented resin canals may be observed traversing the wood for a considerable distance. In both cases, the horizontal canals have their origin in a tangential series of vertically oriented canals of the so-called traumatic type. Similar arcs (fig. 4) or rings of traumatic resin canals and more or less numerous horizontally oriented canals (fig. 3) occur in specimens 2-5 from the auriferous gravels—i.e., in wood from the outer part of the stems of large trees. The occurrence of both vertically and horizontally oriented resin canals of the so-called traumatic type is a characteristic feature of the secondary xylem of *Cedrus* (fig. 2, 5). On the one hand, it serves to differentiate the wood of this genus from that of *Sequoia*, *Abies*, *Tsuga*, *Pseudolarix*, and *Keteleeria*, which do not form extensive horizontally oriented canals; and, on the other hand, from that of *Pinus*, *Picea*, *Larix*, and *Pseudotsuga*, which have vertical canals of the so-called normal or diffused type.⁴

⁴ Thomson and Sifton (1925) and Bannan (1936) have presented evidence which indicates that all the vertical resin canals in the secondary xylem of Pinaceae, regard-

Additional evidence for believing that the five specimens from the auriferous gravels actually are fragments of the wood of *Cedrus* is afforded by the pitting of the tracheids. An alternating arrangement of the bordered pits is of more frequent occurrence in the secondary xylem of *Cedrus* than in that of any other living representative of the Coniferae, exclusive of the Araucariaceae. In the large, thin-walled tracheids of the innermost rings of the root and of the outermost rings of very large, old, slowly-growing stems, numerous transitions occur from an opposite to an alternating arrangement of the bordered pits (fig. 17), with a concomitant elimination of primary pit-areas and crassulae (Bars of Sanio). In other words, the ratios of widely-spaced, compressed, opposite, alternate, and clustered tracheary pitting to one another vary greatly in specimens from different parts of a single tree and from trees grown under different environmental conditions, one type prevailing in one specimen and a different type in another specimen. Similar variations in the tracheary pitting are characteristic of the woods from the auriferous gravels (fig. 16, 18). Furthermore, in the bordered pits of these specimens, as of *Cedrus libanotica* Link., *C. Deodara* Loud., and *C. atlantica* Man., the conspicuously thickened tori (fig. 14, 15) exhibit a coarsely lacerate appearance of unusual constancy, regardless of the part of the plant from which the tracheids are derived and regardless of the presence or absence of primary pit-areas and crassulae.

There is one structural feature, however, in which the wood of the Miocene *Cedrus* differs from that of extant species of the genus. No ray tracheids are present in the Miocene woods. The rays are characterized by having more or less numerous irregularly less of whether they are tangentially aggregated or diffused through the wood, are induced by the effects of abnormal environmental influences. The various representatives of the Coniferae form a graded series of varying sensitivity to abnormal environmental influences; but this sensitivity fluctuates more or less during different stages of ontogeny and under different growth conditions. In exceptional cases, the first-formed annual rings of *Picea* and *Larix* may be devoid of resin canals or have those of the tangentially aggregated type, whereas the subsequently-formed layers of secondary xylem exhibit diffused or scattered canals. Thus, under certain circumstances, small stems or roots of these genera may have an atypical structure which must be taken into consideration in paleobotanical investigations.

Fig. 9-18.—Fig. 9. *Sequoia sempervirens*. Tangential longitudinal section of the xylem, showing ray cells with unevenly thickened primary walls only. Note absence of pitlike depressions directed towards the intercellular spaces. $\times 700$.—Fig. 10. *Keteleeria Davidiana*. Tangential longitudinal section of the xylem, showing in the secondary wall of ray cell (a) four pits oriented towards the four intercellular spaces. These pits are filled with a darkly-colored material. $\times 980$.—Fig. 11. Specimen No. 4. Tangential longitudinal section of the xylem, showing in the secondary wall of ray cell (a) three pits oriented towards intercellular spaces but no pit in the lower right hand corner of the cell. $\times 980$.—Fig. 12. *Keteleeria Davidiana*. Tangential longitudinal section of the xylem, showing vertically oriented parenchymatous cells with both primary (dark) and pitted secondary (light) walls. $\times 980$.—Fig. 13. *Sequoia sempervirens*. Tangential longitudinal section of the xylem, showing wood parenchyma with primary walls only, but having deeply depressed primary pit-areas. $\times 700$.—Fig. 14. *Cedrus Deodara*. Radial longitudinal section of the xylem, showing typical form of the torus. $\times 1850$.—Fig. 15. Specimen No. 4. Radial longitudinal section of the xylem, showing typical form of the torus. $\times 1450$.—Fig. 16. The same, showing opposite tracheary pitting, tori, and crassulae (light). $\times 300$.—Fig. 17. *Cedrus Deodara*. Radial longitudinal section, showing transitions from opposite to alternate tracheary pitting and elimination of crassulae (dark), but not of tori. $\times 370$.—Fig. 18. Specimen No. 4. Radial longitudinal section, showing alternate tracheary pitting. $\times 300$.

shaped, often crystal-bearing, marginal cells (fig. 19), such as occur sporadically in *Keteleeria*, *Abies*, *Pseudolaria*, and even, at times, in existing species of *Cedrus* (Chrystal, 1915). The absence of ray tracheids indicates merely that the Miocene woods were derived from some extinct species of *Cedrus*. It should be emphasized, in this connection, that the woods of various Cretaceous pines similarly are devoid of ray tracheids (Kräusel, 1917) and thus differ from the woods of surviving species of *Pinus*.

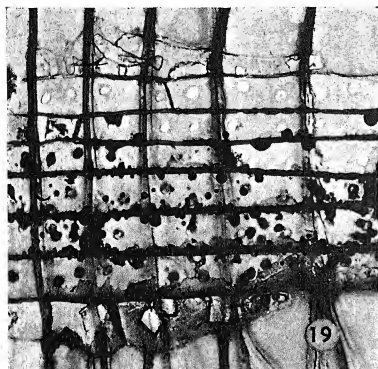


Fig. 19. Specimen No. 4. Radial longitudinal section of the xylem, showing crystal-bearing marginal cells of a ray. $\times 300$.

DISCUSSION.—In dealing with coniferous woods from Mesozoic and Tertiary deposits, paleobotanists are accustomed to refer their specimens to one of various form-genera rather than to specific living genera. The question arises, to which of these form-genera should the woods from the auriferous gravels be referred?

Cedroxylon Kraus, as redefined by Gothan (1905), Kräusel (1917), Torrey (1923) and others, is resorted to for the reception of abietinean woods which are either devoid of resin canals or have canals of the so-called traumatic type only. Various woods of this general structural form, which exhibit an unusually pronounced tendency towards compression or alternation of the tracheary pits, are referred, however, to such form-genera as *Protocedroxylon* Gothan, *Metacedroxylon* Holden, *Planoxylon* Stopes, *Thylloxylen* Gothan, or *Protopiceoxylon* Gothan.

A detailed study of the range of structural variability in *Keteleeria* and *Cedrus* (Bailey, 1933) indicates that samples of the wood from different trees, and even from different parts of the same tree, have combinations of structural characters which closely

resemble those that occur, not only in *Cedroxylon*, but also in one or more of these supposedly transitional Coniferae. Therefore, if *Cedroxylon* is to cover the range of structural variability of such genera as *Abies*, *Tsuga*, *Cedrus*, *Pseudolaria*, and *Keteleeria*, as has frequently been assumed, it must obviously be redefined in such a manner as to obviate the necessity for several of the form-genera which are listed in the preceding paragraph. Such paradoxical situations as these in the identification and classification of coniferous woods are due to a lack of comprehensive and reliable information regarding the limits of variability of anatomical features in the Coniferae, both living and extinct. They can not be fully clarified until large collections of authentic specimens are assembled not only from different genera and species but also from different parts of the tree and from trees grown under widely divergent environmental conditions.

It seems advisable, accordingly, to refer the woods from the auriferous gravels to *Cedrus* rather than at this time to attempt a premature revision of *Cedroxylon* or of some other form-genus for their reception. Specimen No. 7354 in the paleobotanical collections of Harvard University was originally described by Jeffrey (1904) as *Sequoia Penhallowii* and, therefore, becomes the type of *Cedrus Penhallowii* (Jeffrey) n. comb.

SUMMARY

The auriferous gravels of California frequently contain logs and smaller fragments of wood which are not mineralized, lignitized, or carbonized, yet exhibit such a remarkable state of preservation after millions of years that they have aroused much interest among both laymen and scientists.

Many of the specimens exhibit superficial resemblances in color and texture to the wood of *Sequoia*, to which genus they have commonly been assigned.

A detailed study of such specimens from Eldorado, Placer, and Yuba counties indicates that the woods are derived from at least three different genera—viz., *Sequoia*, *Libocedrus*, and *Cedrus*.

The occurrence of the wood of *Cedrus* is particularly interesting, since this genus has not been recorded previously from the Cretaceous or Tertiary deposits of western North America.

The wood from the Miocene auriferous gravels closely resembles that of *Cedrus libanotica*, *C. Deodara*, and *C. atlantica*, but may be differentiated from that of these surviving species by the absence of ray tracheids.

The Miocene woods are referred to *Cedrus Penhallowii* (Jeffrey) n. comb., the type specimen of which is No. 7354 in the paleobotanical collections of Harvard University.

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SECTIONAL ISSUANCE OF BIOLOGICAL ABSTRACTS—PLAN FOR 1939

FIFTEEN YEARS ago American biologists became convinced that the mounting periodical literature of the subject had to be brought under better control. It was more than any library could acquire, more than any person could read. Hence the organization of *Biological Abstracts*, now in its twelfth annual volume. However, this publication has a fatal weakness. The Union of American Biological Societies that gave it being was only a paper union. There was neither association nor revenue.

Thanks to mighty friends it found, outstanding among these the Rockefeller Foundation which contributed nearly three quarters of a million dollars, *Biological Abstracts* had a period of maximum prosperity. But in the end there were only 2000 subscribers, two-thirds of them institutional; just enough to meet 40 per cent of the outlay. With the necessary close of Foundation financing in 1937 the problem became acute. The generosity of subsidizing institutions and fine patronage from abroad, under an emergency arrangement of scaled charges, has prevented suspension during 1938 and provided the opportunity of working out a permanent plan for 1939. Such a plan is here presented in the confident belief that it will give general satisfaction, make the *Abstracts* fully self-supporting and enable it to extend its service.

One of the prime objectives in the initiation and development of *Biological Abstracts* has related to its important rôle in integrating the findings of science in an age where specialization is the watchword. In

spite of this rather obvious and generally desirable goal there has been a constant undercurrent, and in some quarters a rather insistent demand, for a return to the specialized type of abstract journals, or for a printing of *Biological Abstracts* in separate parts so that opportunity would be afforded those so desiring to subscribe to the group of subjects in which they, individually, were most interested. Many reasons for this viewpoint have, from time to time, been offered, chief among them being a logically assumed reduction in cost for a part as compared with the whole.

Both these viewpoints are perfectly legitimate and understandable, and the problems thereby presented are surely worthy of every effort toward ultimate solution—so long as the general end in view is the highest good to the greatest number.

In the endeavor to meet the situation satisfactorily, a plan has been evolved whereby *Biological Abstracts*, while continuing to appear as a single complete entity, might, without repaging or other change except in the cover, be also broken up into several subject groups which would be made available to individuals not desiring the complete service. The idea seems practicable, and the following subject grouping into five "reprint" parts is adopted for 1939.

I. *Abstracts of General Biology* to include General Biology, Biography-History, Bibliography, Evolution, Cytology, Genetics, Biometry, and Ecology; price \$4.

II. *Abstracts of Experimental Animal Biology* to include Animal Physiology, Nutrition, Pharmacology, Pathology, Anatomy, Embryology, and Animal Production; \$9.

III. *Abstracts of Microbiology and Parasitology* to include Immunology, Bacteriology, Viruses, Parasitology, Protozoology, and Helminthology; \$5.

IV. *Abstracts of Plant Sciences* to include Phytopathology, Plant Physiology, Plant Anatomy, Paleobotany, Systematic Botany, Agronomy, Horticulture, Forestry, Pharmacognosy, and Pharmaceutical Botany; \$6.

V. *Abstracts of Animal Sciences* to include Paleozoology, Parasitology, Protozoology, and Helminthology, Systematic Zoology, and Economic Entomology; \$6.

The subscription price for the complete volume 13 for 1939 will be \$25 to individual subscribers and institutions alike.

A plan of this nature would involve merely the lifting out (without resetting of type or renumbering of the abstracts) of the sections appertaining to the subject group concerned and binding them in a separate cover which would bear the appropriate subtitle. This procedure would undoubtedly be facilitated by rearrangement of some of the sections. The complete index, going to *all* subscribers, would still preserve most of the desirable long-range features of the full service, and especially so since all subscribing libraries and institutions will, of course, want *Biological Abstracts* in its entirety. This would meet the requirements alike of specialization and general survey at prices that will be found feasible by both biologists and libraries. It is most emphatically not proposed to destroy *Biological Abstracts* by breaking it up into its components, but rather to maintain it as

an integral unit while offering the membership of the separate societies many of the advantages which they would have if each society had its own individual abstracting journal.

One thing is already assured. Coverage will be prompt, as also the issue of indexes. This year has proved that. The aim is a lag of no more than two months, and index in the spring. The extent of coverage must, of course, depend on the extent of support. Biologists can get only what they pay for. With no national society treasury to draw upon, this must be so. At least 1250 subscriptions to the complete edition, and four times as many orders for section issues, are required to make possible publication without deficit. Libraries can be depended on to provide the former. Is it too much to expect an average of 1000 individual subscribers to each section?

Each of the biological societies concerned is being invited to set up a committee which will be effective in fixing the editorial policies and standards of the *Abstracts* within its respective field.

In order to facilitate the plans for 1939, subscription blanks will be shortly distributed to the entire membership of the Societies constituting the Union as well as to libraries and other institutions far and wide. It is hoped that as many responses as possible may be forwarded by November 1 to the Office of the Business Manager, Biological Abstracts, University of Pennsylvania, Philadelphia, Pennsylvania.

Board of Trustees of *Biological Abstracts*.

September 20, 1938.

APHANOMYCES PHYCOPHILUS IN CULTURE¹

Alma J. Whiffen

APHANOMYCES PHYCOPHILUS De Bary, one of the rarer Saprolegniales, was first described by DeBary in 1860 from a collection made at Frankfurt am Main. Since then it has been reported from Michigan by Kauffman (1915), from Indiana by Weatherwax (1914), from New York by Sparrow (1933), and from North Carolina by Couch (1926). Previous attempts to obtain saprophytic growth of this parasite have been unsuccessful. DeBary says that it is parasitic on *Spirogyra* and *Zygnema* and is not to be cultured on decaying Phanerogam remains and dead animals. The purpose of this paper is to describe experiments in which it has been possible to induce growth of *Aphanomyces phycophilus* on artificial culture media.

GROWTH ON SPIROGYRA.—The hyphae, 8–15 μ thick, grow lengthwise through the *Spirogyra* filament by penetration of the cross walls. The tip of the growing hypha upon coming in contact with the cross wall of the host becomes somewhat flattened and presses against it as the wall becomes thinner. When a hole has been dissolved in the wall, the hypha pushes through it rapidly into the adjoining cell, which becomes plasmolysed. One hypha was seen to grow through one cell, a distance of 150 μ , in 20 minutes. After traversing the length of the cell along the surface of the plasmolysed protoplast, the hypha sends down lateral branches among the chloroplasts. The main hypha may continue on into the next cell, or a lateral branch may penetrate the side wall and grow out some distance into the water and parasitize another *Spirogyra* filament. Oogonia, 40–50 μ in diameter, are formed on the ends of short lateral branches and seem to be intramatrical as often as extramatrical. The oogonial wall is drawn out into a number of spines, 7–9 μ in length, and is yellow-brown in color. The single brownish oospore, 24–31 μ in diameter, is finely granular, with one or two rows of oil globules around the periphery. The curved-clavate antheridia develop on the ends of nearby lateral branches and often wind around the hyphae bearing oogonia. There are one to three antheridia on each oogonium. No spores were observed.

ISOLATION AND CULTURE ON AGAR.—A fungus was found growing parasitically on *Spirogyra* in a collection made by Mrs. H. B. Gotass on April 16, 1938, from a small stream near Chapel Hill. The size of the hyphae, the mode of growth, the structure of the oogonia, and the antheridia all agreed with DeBary's description and figures of *Aphanomyces phycophilus*. It differed from Wille's (1899) description of *A. nor-*

vegicus in having the hyphae running inside the host cells rather than along the outer surface of the wall.

The fungus was isolated by placing parasitized filaments of *Spirogyra* on the surface of plain 2 per cent agar in a Petri dish. By the following day the fungal hyphae had grown out sufficiently over the surface and through the agar to be cut out and transferred to a plate of nutritive agar. Transfers were made to corn meal agar, to corn meal agar to which had been added Fleischmann's yeast in the proportion of one fourth of a cake to 100 cc. of agar, and to a maltose-peptone agar, which is designated as M. P. No. 5 agar in our laboratory and consists of 3 g. maltose and 1 g. peptone in 1000 cc. of 2 per cent agar. No growth was made on the corn meal agar, but some growth occurred on the yeast-corn meal agar. The most vigorous growth, however, was obtained on the maltose-peptone agar, and this agar has been used in maintaining our culture of *A. phycophilus*. Blakeslee's No. 230 agar and a potato-dextrose agar were tried with no growth on the No. 230 and with but poor growth on the potato-dextrose agar. This fungus would not grow on hemp seeds in water. Oogonia and antheridia were produced in large numbers in the maltose-peptone agar, but the eggs did not mature. On agar the oogonial wall was almost hyaline. The oospores, however, matured when some of the fungal hyphae in agar were transferred to distilled water. It was also found possible to infect healthy *Spirogyra* threads by placing them in water over a small cube of agar containing hyphae of the fungus, and oogonia developed which matured normally and were yellow-brown in color. In one instance *Spirogyra* was inoculated with the parasite in the morning, and by 5 p.m. the fungal hyphae had begun growing within the host filaments. By 3:30 the following afternoon young oogonia were formed. The parasite was also induced to grow on two other species of *Spirogyra*, but infection was slower. Attempts to inoculate *Oedogonium*, *Vaucheria*, and *Chara* failed.

EXPERIMENTS WITH VITAMIN B₁.—To increase growth, varying amounts of vitamin B₁, in aqueous solution, were added to the maltose-peptone agar. The unit of vitamin chosen is the same as that employed by Robbins and Kavanagh (1938) in their growth experiments with vitamin B₁ and is equal to 10⁻⁹ mole or .000000346 g. of Betabion. The vitamin was added in proportions of 100, 10, 1, .10, and .01 units to each 10 cc. of agar. The expansion of the culture on the agar containing 100 units of vitamin B₁ after five days was more than twice as much as that made on the agar with .01 unit. Hyphae in the agar with 100 units of Betabion grew to a maximum size of 30 μ in diameter, twice that of the largest normal hyphae.

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This study was suggested by and carried out under the direction of Dr. J. N. Couch to whom the writer expresses her sincere gratitude and appreciation. To Dr. J. E. Adams is also extended appreciation for assistance.

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DESCRIPTION OF GROWTH ON MALTOSE-PEPTONE NO. 5 AGAR.—The growth of *A. phycophilus* on agar does not at all resemble that made by other species of *Aphanomyces* as *A. stellatus*. Growth is slow, filling the Petri dish after about twelve days. Under low power the culture has a much tangled appearance with a few large hyphae crossing in all directions. These main hyphae send out at right angles a profusion of lateral branches which terminate in digitate

pseudo-haustoria. Some hyphae may grow up into the air. The protoplasm is coarsely granular and often intermingled with droplets of oil, especially in the older portions where there is little cytoplasm.

Further study into the cytology of *A. phycophilus* might prove interesting in regard to its taxonomic position. Though the egg structure of *A. phycophilus* is similar to that of the genus *Aphanomyces* (see Coker, 1923), the size of the hyphae and the mode of growth on agar would seem to indicate that *A. phycophilus* does not correctly belong in this genus. The production of sporangia and spores would throw much light on the problem, but so far in the present study no spores have been observed.

SUMMARY

Aphanomyces phycophilus, which previously has been considered an obligate parasite, for the first time has been cultured on nutritive agar—a maltose-peptone agar giving the best results.

The addition of vitamin B₁ to the culture medium increased growth.

Growth on agar is characterized by the termination of lateral branches in digitate pseudo-haustoria and is different from that of any other species of *Aphanomyces*.

It is suggested that further studies on this fungus might show that it does not belong in the genus *Aphanomyces*.

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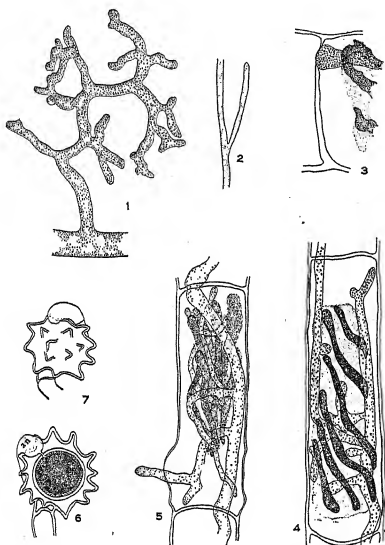


Fig. 1-7. Fig. 1. Main hypha, lateral branch, and pseudo-haustoria of *A. phycophilus* from a culture on M. P. No. 5 agar. $\times 330$.—Fig. 2. Main hypha and lateral branch of *A. stellatus* from a culture on M. P. No. 5 agar. $\times 330$.—Fig. 3. Tip of growing hypha about to push through cross wall of *Spirogyra* cell. $\times 330$.—Fig. 4. Parasitized cell of *Spirogyra* in which the main hypha has ceased to elongate and one of the lateral branches has entered the adjoining cell. $\times 180$.—Fig. 5. Parasitized cell of *Spirogyra* showing typical twisting of the fungal hypha at the cross wall and a lateral branch leaving the cell. $\times 180$.—Fig. 6. Antheridium and oogonium showing structure of oospore. $\times 330$.—Fig. 7. Surface view of antheridium and oogonium. $\times 330$.

REDUCTION DIVISIONS IN TRIPLOID IMPATIENS¹

Frank H. Smith

THE AUTHOR recently reported that certain species of *Impatiens* invariably show prochromosomes in metabolic nuclei, but that in the nuclei of other species of this genus these structures are absent (Smith, 1934). Following this study, attempts were made to cross species showing prochromosomes with species not possessing such structures in order to determine the mode of inheritance of this nuclear character. Four species in which prochromosomes occur (*I. pallida*, *I. biflora*, *I. Balsamina*, and *I. Roylei*) were used first as female parents, and each of these was pollinated successively with pollen from three species (*I. scabrida*, *I. sultanii*, and *I. Holstii*) in which prochromosomes are absent. Reciprocal crosses between these species were also attempted. No hybrids were obtained, however, with any of these cross-pollinations. At least twenty pollinations were completed for each of the above-mentioned crosses. Hence it would appear that these species can be hybridized only with difficulty or not at all.

It must be admitted that the technique used in pollinating the stigma was not perfect. It is necessary in *Impatiens* to emasculate the flowers some time before they open because of the early maturation of the anthers. This almost invariably results in so much injury to the tightly folded corolla that it withers and soon falls, and frequently the entire flower dies soon after emasculation. Also it is very difficult to remove the anthers intact, since they are united around the ovary and during the earlier stages are united with the style. From a few pollinations seeds were obtained which were germinated, and the chromosomes were examined in the seedling root tips. All the seedlings, with one exception, had the diploid complement of chromosomes of the female parent. Obviously the seeds were produced by self-pollination resulting from the rupturing of the anthers while removing them from the flower.

One seedling was found to be a triploid form of *Impatiens Balsamina*. The seed which produced this triploid plant was formed as a result of an attempted cross between *I. Balsamina* and *I. sultanii*, the latter species supplying the pollen. *I. Balsamina* has fourteen comparatively small chromosomes in the root tips (fig. 1), while *I. sultanii* has sixteen chromosomes which are considerably larger (fig. 2). The satellite chromosomes in the latter species have an almost median spindle-fiber constriction, whereas in *I. Balsamina* the constriction is considerably closer to the end of the chromosome bearing the satellite. In the ovary involved in this particular pollination five seeds were produced, all of which appeared to be typical seeds of the female parent. When the seedling root tips were examined, four of the plants proved to be diploid *I. Balsamina*, while the fifth was obviously a triploid form of the same species. The cells con-

tained twenty-one chromosomes (fig. 3), and it was readily discernible that they represented three sets of chromosomes from *I. Balsamina*. This was shown especially by the morphology of the satellite chromosomes. Since most triploids probably occur as a result of the fertilization of a diploid egg by a haploid male gamete, the most likely assumption in this particular case is that an egg with the diploid number of chromosomes was present and that broken anthers resulted in self-pollination.

The seed germinated as readily as the seeds with normal embryos from the same ovary. The mature plant had very much the same general appearance as one of a diploid *I. Balsamina*, except that it was perhaps a little more vigorous and that the swellings at the nodes were noticeably larger than those of the diploid. Cuttings were readily produced, so that several triploid plants were available for this study.

Young anthers were removed from the perianth, treated for a short time with Carnoy's solution (6 parts absolute alcohol, 3 parts chloroform, 1 part glacial acetic acid) and fixed in Karpechenko's modification of Navashin's solution. The usual paraffin technique was used, and the sections were stained with the Gram stain in combination with picric acid.

THE REDUCTION DIVISIONS.—The reduction divisions proceed regularly up to late diakinesis or the equatorial-plate stage. During diakinesis the chromosomes are usually associated in seven sets of trivalents (fig. 4). Usually the chromosomes of each set are combined in a chain. In some cases two of the chromosomes are paired side by side, and the third member of the trivalent is attached to one end of these at the point of union. In other trivalents all three chromosomes are attached at one end of each. In all probability synapsis always takes place to some extent between the three homologous chromosomes, since in no case was a univalent observed during early diakinesis.

In late diakinesis, especially after the nuclear membrane begins to disappear, there is a tendency for the chromosomes of a trivalent to separate. Invariably all twenty-one chromosomes become arranged on the same spindle, and no secondary spindles such as McClintock (1929) found in triploid corn were observed. An analysis of 791 figures of late diakinesis and pre-equatorial-plate gave the results listed in table 1. These configurations are illustrated by figures 5-10.

TABLE 1.

Arrangement of chromosomes	7'''	6'''	5'''	4'''	3'''	6'''
		1''	2''	3''	4''	
		1'	2'	3'	4'	3'
Number of cells	391	242	122	32	3	1

¹ Received for publication June 24, 1938.

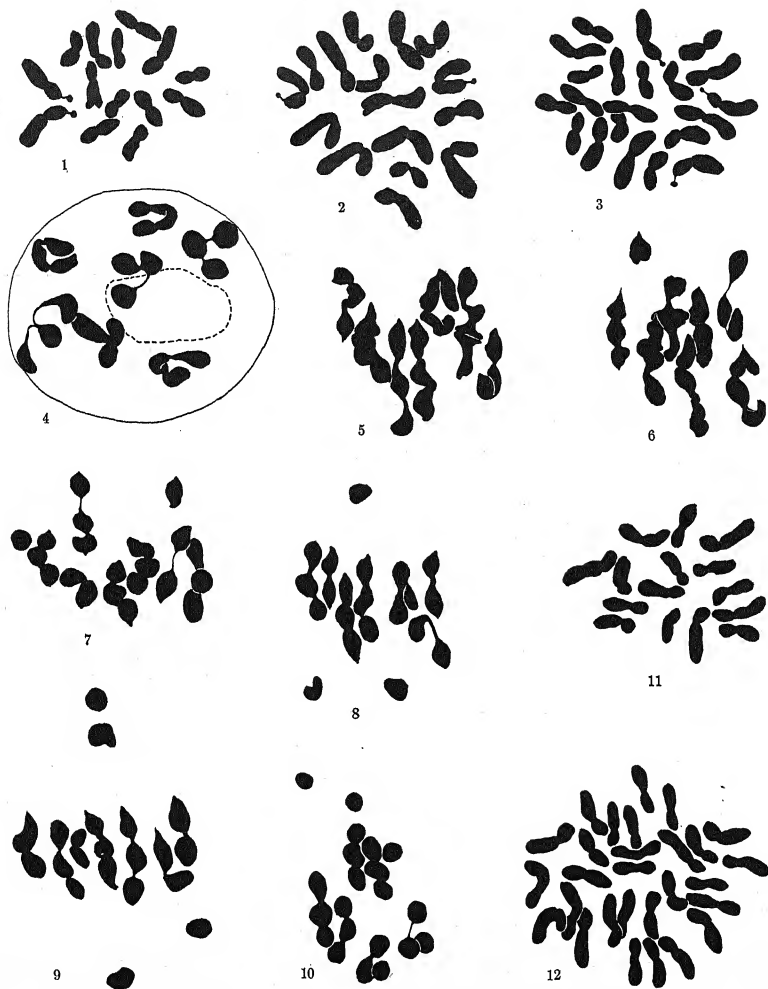


Fig. 1-12. All figures were drawn with the aid of a camera lucida at a magnification of approximately 4000 diameters and have not been reduced in publication.—Fig. 1. Somatic plate of *I. Balsamina*.—Fig. 2. Somatic plate of *I. sultani*.—Fig. 3. Somatic plate of triploid *I. Balsamina*.—Fig. 4. Nucleus of triploid sporocyte at diakinesis showing seven trivalents.—Fig. 5. Metaphase with seven trivalents.—Fig. 6. Metaphase with six trivalents, one bivalent, and one univalent.—Fig. 7. Pre-equatorial-plate with five trivalents, two bivalents, and two univalents.—Fig. 8. Metaphase with four trivalents, three bivalents, and three univalents.—Fig. 9. Metaphase with three trivalents, four bivalents, and four univalents.—Fig. 10. Pre-equatorial-plate with six trivalents and three univalents.—Fig. 11. Somatic plate of trisomic *I. Balsamina*.—Fig. 12. Somatic plate from a tetraploid root tip.

It is not possible satisfactorily to identify individual sets of chromosomes in this triploid during the reduction divisions. It is possible of course for configurations other than those listed in table 1 to occur, but such configurations were not observed. In the majority of cases no univalents or only one or two were found in each pollen mother cell. It is doubtful if more than four univalents were produced in any one of the pollen mother cells in this triploid. This would indicate again that synapsis usually occurs between the three homologous chromosomes in each trivalent.

During the metaphase stages the bivalents and trivalents are usually arranged fairly regularly in the equatorial-plate region (fig. 5-10). The bivalents take up a typical position at this time and separate regularly during the anaphases. The usual position of the trivalents is in a chain of three chromosomes arranged parallel to the axis of the spindle. During the anaphases an end chromosome from this type of configuration moves to one pole, and the remaining two chromosomes move to the opposite pole of the spindle. The latter two chromosomes become separated during the passage to the pole. In some cases the trivalent is arranged in a curve in the equatorial-plate region, with the two end chromosomes directed toward one pole and the middle chromosome toward the other. From this arrangement the middle chromosome moves in one direction during the anaphases,

chromosome being left out of the spindle entirely. In still fewer instances the two chromatids of a univalent chromosome separate during the anaphases of the heterotypic division. If such a separation occurs at this time, no split is evident in the second division. Thus at times the number of chromosomes at the two poles of a homeotypic spindle are not equal. Micro-nuclei such as have been reported by McClintock (1929) for triploid corn and by Woods (1937) for triploid tulip were rarely observed. In this respect the behavior is similar to that in the triploid *Petunia* described by Steere (1932).

Aside from the irregularity in number mentioned above, the homeotypic divisions proceed normally. The chromosomes are arranged regularly on the plate, the chromatids separate in the usual manner and move to the poles without any lagging or fragmentation. There is no loss of chromosomes during the homeotypic divisions, and four microspores are produced from practically all sporocytes. Approximately half of these develop into pollen grains that are well filled and appear as if they should be functional. The remainder of the young microspores collapse soon after the tetrad stage.

PROGENY OF THE TRIPLOID.—In spite of the occurrence of numerous well filled pollen grains, the triploid proved to be very highly self-sterile. Only one seed was produced from many self-pollinations. This

TABLE 2.

Distribution	10-11	9-12	8-13	7-14	10½-10½	9½-11½	10-10	9-11
Frequency	210	83	21	3	2	2	8	2

and the two end chromosomes move in the opposite direction. It is very seldom that a single chromosome is left on the equatorial plate. In such cases the chromosome is lost in the cytoplasm.

The univalent chromosomes are usually found somewhere on the spindle between the plate and one of the poles. It is seldom that a univalent is found exactly on the plate. If one does take up such a position, the two chromatids usually separate during the heterotypic division. The scattered univalents move to the poles early, and in some cases single chromosomes are found at the poles before any disjunction has occurred of the bivalents and trivalents on the plate.

The separation of the chromosomes during the anaphases results in a random distribution of the chromosomes to the poles. Hence the most common distribution is ten to one pole and eleven to the other. Table 2 gives the frequency of various distributions observed in 331 pollen mother cells in which both groups of anaphase chromosomes could be counted.

It will be noticed that in only a small fraction of the cases is a univalent lost in the cytoplasm. This results either from a chromosome being left at the plate during the anaphases or more rarely from a

seed germinated, but the seedling did not survive, and no chromosome counts are available for it.

The triploid was highly sterile also when pollinated with pollen from a diploid plant of *I. Balsamina*. Only two seeds were produced from about twenty-five pollinations. When root tips from the seedlings were examined, it was found that one of the resultant plants was a diploid and the other a trisomic with fifteen chromosomes (fig. 11).

Four seedlings were produced from seeds resulting from twenty-five pollinations of a diploid plant with pollen from the triploid. Chromosome counts made from the root tips of these seedlings were very confusing. On each plant there were some root tips which were entirely tetraploid, each cell containing twenty-eight chromosomes (fig. 12). The cells in other root tips showed the normal diploid complement. These seedlings were transplanted twice before root tips were taken for study. It is possible that injuries to the root system resulted in the formation of callus tissue and the consequent production of tetraploid roots on a diploid plant. This would be comparable to the production of tetraploid stems from callus tissue such as has been reported in tomato by Lindstrom and Koos (1931) and others. Unfortunately for this study, it was necessary to transport

these seedlings and the trisomic plant resulting from the triploid \times diploid pollinations across the country, and they died in transit. Hence a complete analysis could not be made of these seedlings.

SUMMARY

An autotriploid of *Impatiens Balsamina* was obtained accidentally from an attempted cross between the above species and *I. sultani*.

Synapsis was fairly complete, and during early diakinesis the chromosomes in practically all pollen mother cells were associated in seven sets of trivalents. During late diakinesis and pre-equatorial-plate there is a tendency for one chromosome to separate from each trivalent, but in most pollen mother cells there are no univalents produced at this time or only one or two. In no sporocytes were more than four univalents observed.

The chromosomes are arranged fairly regularly on a single spindle during the heterotypic division, and disjunction occurs at random with regard to each trivalent. The homoecotypic divisions are uniformly regular. There is a notable lack of lagging chromo-

somes or of fragmentation, and micro-nuclei or micro-pollen grains are rarely produced.

The triploid was extremely sterile both when selfed and when crossed with a diploid plant.

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A QUANTITATIVE STUDY OF THE SUBTERRANEAN MEMBERS OF THREE FIELD GRASSES¹

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IN A previous paper the writer (Dittmer, 1937) recorded his measurements of both roots and root hairs on a single winter rye plant. From careful counts of extensive samplings it was determined that this plant had approximately 13,800,000 roots with a total length of over 385 miles and a surface area of about 2,550 square feet. The root hairs of this plant numbered approximately 14,000,000,000 with a total length of over 6,600 miles and a surface area of about 4,320 square feet. Attention was drawn to the significant role such a fibrous root system must play in soil physics and in soil binding. Important studies by Weaver (1925, 1926), Kieselbach (1910), and others were reviewed. The quantitative data relating to the roots of certain grains, reported by Pavlychenko (1937), were summarized.

The present paper gives the results of a comparative study of roots and root hairs of *Poa pratensis* L., *Secale cereale* L., and *Avena sativa* L. These plants when surveyed were fruiting but still green and had grown outdoors under field cultivation. Soil samples were limited to the upper six inches, since it is in this zone that roots and root hairs of these plants grow most abundantly. Both the extent and the massing of subterranean members in upper soil levels have given grasses peculiar importance in soil conservation work.

¹ Received for publication June 24, 1938.

The writer wishes to express his sincere appreciation to Professor R. B. Wylie, of the State University of Iowa, under whose direction this work was done.

METHOD.—Soil samples 3 inches in diameter and 6 inches deep (42 cubic inches) were taken in the field by means of a cutting tube. The tool used was an iron pipe of 3 inches inside diameter and 4 feet long. At one end a cutting edge had been ground with the taper on the outside to prevent soil packing. Beginning three inches back of the cutting edge a section one inch wide was cut out to facilitate freeing the collected samples from the tube. This was placed over selected soil areas and driven into the ground to a depth of six inches. After the samples were flushed from the pipe and the soil washed from the roots, they were preserved for study in a 5 per cent solution of formalin. Three soil samples each for oats, winter rye, and Kentucky bluegrass were surveyed and the measurements tabulated.

Counts were made of both attached roots, included in the soil samples, and portions cut off from adjacent plants. All main roots, which were readily identified by their size, were counted and measured. Fifty of these roots, complete with their branches, were then selected at random and counts made to determine the average number of secondaries and tertiaries per main root. Although the larger roots could be measured directly, it was necessary to use a biobjective binocular to determine accurately the number and lengths of the small tertiary roots. The diameters of all root members were measured with the aid of a compound microscope fitted with ocular micrometer.

TABLE 1. *Roots. Quantitative analysis of the roots; values given are the average for three soil samples each 3 inches in diameter and 6 inches deep (42 cubic inches).*

Root categories	Total no. of roots	Av. root length (mm.)	Total length of roots (feet)	Av. diam. of roots (microns)	Total root surface (sq. in.)	Volume in cubic mm.
<i>Avena sativa</i>						
Main	110	81.0	30	510	23	1,920
Secondary	2,190	11.1	80	180	22	620
Tertiary	2,400	4.8	40	90	5	700
Totals	4,700		150		50	2,610
<i>Secale cereale</i>						
Main	130	99.0	40	570	35.0	3,300
Secondary	3,670	10.6	130	195	37.0	1,180
Tertiary	2,600	4.3	40	105	5.9	100
Totals	6,400		210		77.9	4,580
<i>Poa pratensis</i>						
Main	900	64.0	180	480	134	9,750
Secondary	29,700	6.6	870	135	173	3,800
Tertiary	43,900	1.4	200	85	25	350
Totals	84,500		1,250		332	13,900

Root hair counts and measurements were subsequently made for representatives of all categories, following the method previously described by the writer (Dittmer, 1937). Root hairs on one-third of the roots, selected by random sampling, were counted and measured and these data used in calculating the totals for a given soil sample. Since the figures presented in the following tables are the results of calculations based on the samples counted and measured, they are recorded in the nearest round numbers.

INTERPRETATION OF RESULTS. — Winter rye, compared with oats, had approximately 50 per cent more roots, with about 25 per cent greater total length and 35 per cent more surface. However, Kentucky bluegrass, compared with rye, had more than 12 times as many roots, with 6 times the root length and 5 times the surface area. Roots of the secondary category had the greatest length and largest surface area in all three grasses. The extensive subterranean growth of bluegrass in upper soil levels was due mainly to the large number of plants per unit area resulting from its propagation by means of rhizomes. The rhizomes of this species had alone a total length of 24 inches per sample volume and carried about 12 per cent of the roots.

Living root hairs were scattered over the entire surface of all roots of the three species studied. A few older roots of Kentucky bluegrass had lost their hairs, but these were dark brown in color and apparently no longer functional. Considering the number of root hairs for oats, winter rye, and bluegrass, the comparative totals were as 1, 2, and 8, respectively. For these three grasses their totals for root hair length were as 1, 2, and 6, and their total exposed surfaces as 1, 2, and 4, respectively. It may be noted that the relations to all totals remained the same for oats and rye. However, root hairs of bluegrass, com-

pared with those of oats, had 400 per cent more surface area, and 800 per cent greater number.

In view of their extent and surface area, it would seem that the subterranean members of these grasses should occupy much of the soil volume. However, roots and root hairs of oats occupied but 0.55 per cent of their total soil volume. Roots alone accounted for over two-thirds of this total, whereas root hairs which exposed 11 times as much surface as the roots occupied less than one-third of the volume of the underground plant parts. Roots and root hairs of winter rye together occupied 0.85 per cent of its soil volume, but the root hairs, which exposed 15 times more surface than the roots, occupied only one-half of their volume. Subterranean members of Kentucky bluegrass occupied 2.8 per cent of the soil volume, and the root hairs accounted for less than one-fourth of this total, although they exposed 7 times as much surface as the roots.

Assuming that roots and root hairs were evenly distributed throughout the samples, one cubic inch of soil would have approximately 110 oat roots and 150,000 root hairs, with a combined length of about 630 feet and a surface area of 15 square inches. A similar cube of soil from a field of winter rye would have approximately 150 roots and 300,000 root hairs, with a combined length of 1,300 feet and a surface of about 30 square inches. Kentucky bluegrass would have, per cubic inch of soil, approximately 2,000 roots and 1,000,000 root hairs, with a combined length of over 4,000 feet and a surface area of about 65 square inches. Since these subterranean members are relatively fewer in uppermost layers of soil, it follows that in zones of optimum development values would greatly exceed those noted above. When it is considered that these grasses have from 150,000 to 1,000,000 root hairs per cubic inch of soil, their importance in the physics of the soil is obvious.

TABLE 2. *Root Hairs.* Quantitative analysis of root hairs; values given are the average for three soil samples each 3 inches in diameter and 6 inches deep (42 cubic inches).

Root categories	No. of hairs per mm. of root length	Total no. of root hairs (in millions)	Average length of root hairs (microns)	Total length of root hairs (feet)	Av. diam. of root hairs (microns)	Total root hair surface (sq. inches)	Volume in cubic mm.
<i>Avena sativa</i>							
Main	400	3.6	1,400	17,000	14	352	790
Secondary	90	2.2	1,100	7,900	13	154	310
Tertiary	40	0.5	880	1,400	13	27	60
Totals		6.3		26,300 (4.9 miles)		533 (3.7 sq. ft.)	1,160
<i>Secale cereale</i>							
Main	507	6.4	1,720	37,000	15	888.9	1,950
Secondary	135	5.3	940	16,500	12	272.4	550
Tertiary	73	0.8	590	1,600	12	29.0	50
Totals		12.5		55,100 (10.4 miles)		1,190.3 (8.2 sq. ft.)	2,550
<i>Poa pratensis</i>							
Main	480	25.9	1,115	97,000	11	1,528	2,700
Secondary	88	22.8	935	67,400	9	870	1,350
Tertiary	47	2.8	510	4,600	7	49	50
Totals		51.5		169,000 (32 miles)		2,447 (16.9 sq. ft.)	4,100

Discussion.—Competition obviously greatly influences root development for a given grass. In a previous paper the writer (Dittner, 1937) reported for a single winter rye plant, grown in the greenhouse, a total root length of approximately 385 miles. In this study soil samples of competitive field plants indicated a root system about one per cent that of the greenhouse plant of the same species grown without competition. This is in close agreement with the data published by Paviychenko (1937). He found that the root system of a wild oats plant grown out of doors in competition had a total length of 0.6 mile while that of a nearby plant grown free of competition measured 54.3 miles, or a ratio of about 1:110. Since Paviychenko worked with the entire root systems and in the writer's later study data were derived from soil samples, these results suggest that samplings from upper soil levels are a reliable index to the amount of subterranean development for plants of such growth habits.

The number of root hairs also differed considerably for plants grown in the greenhouse and those grown under field conditions. There were 10 times as many root hairs per unit of length on main roots of field rye as on the indoor plants, while the secondary roots on outdoor plants had 3 times as many, and the tertiary roots had twice as many. Snow (1905), working with *Zea Mays* and *Pisum sativum*, and Roberts (1916), who also studied both monocotyledonous and dicotyledonous plants, state that root hair production is in inverse proportion to the elongation of the epidermal cells. They maintain that root hairs will be produced on practically all epidermal cells if these cells are "suppressed." They

attribute this to diminished oxygen supply low temperature, greater abundance of water, or increase of osmotic pressure in the adjoining cortical cells which would then absorb water from the epidermal cells retarding their growth. Snow and Roberts show also that on roots which elongate greatly the epidermal cells are much longer, and many do not produce hairs. The results of this experiment are in harmony with those just reviewed, since the shorter roots of the field plants, grown under competition, branching only within the tertiary order, produced far more hairs per unit of root length than did the indoor plant. However, the total number of root hairs was probably greater on the indoor plant because of the extensive root system including the well developed members of the quaternary division.

From the theoretical standpoint of their usefulness as soil binders these three grasses differ widely. This study indicates that oat would be least efficient in retarding erosion, since it has the least subterranean growth of the three species studied. Moreover, it is a short-lived annual, living but three months, and does not establish a very compact root system. Winter rye, also an annual, is planted in late summer and lives 9-10 months, including the winter period. It exposes a much greater subterranean surface and gives soil protection from autumn to midsummer of the following year and is especially helpful in our area in lessening erosion by early spring rains.

Kentucky bluegrass because of its ready propagation by means of rhizomes forms a very compact vegetative cover. As a perennial it protects the soil 12 months of the year, and throughout the growing season is developing new plants, new roots, and new

rhizomes. This results in an even distribution of plants and a dense turf which provides a relatively permanent soil cover. But it is apparently primarily because of its elaborate subterranean system that bluegrass is so efficient in retarding erosion. Kentucky bluegrass, in areas of its favorable growth, more nearly approaches the ideal plant cover than any other readily cultivated plant.

SUMMARY

A quantitative study was made of the roots and root hairs of oats, winter rye, and Kentucky bluegrass, based on samplings from fields under normal cultivation. Sample volumes of soil 3 inches in diameter and 6 inches deep (42 cubic inches) were removed by means of a cutting tube, and counts and measurements were made of the included plant parts.

Assuming that their roots and root hairs were evenly distributed in the samples in upper soil levels, oats would expose a surface of 15 square inches per cubic inch of soil, rye 30, and bluegrass about 65 square inches. Considering the number of root hairs per cubic inch of soil, oats would have approximately 150,000, rye 300,000, and bluegrass about 1,000,000.

From a practical viewpoint these measurements suggest that oats would be least efficient of the three

grasses, winter rye intermediate, and Kentucky bluegrass far superior to either of the others in soil binding possibilities.

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THE BRAZILIAN MYXOPHYCEAE. II¹

Francis Drouet

THESE STUDIES in the blue-green algal flora of Brazil were initiated in 1935 while I was engaged as Visiting Botanist to the Comissão Técnica de Piscicultura do Nordeste. An account of my own collecting in Brazil, reference to previous physiological work in that country and a general treatment of the Oscillatoriaceae will be found in my first article of this series, 'The Brazilian Myxophyceae. I,' Amer. Jour. Bot. 24: 598-608 (1937).

It has been my good fortune, through the kindness of Messrs. J. Ramsbottom and G. Tandy, of Dr. Th. Arwidsson, Dr. O. C. Schmidt, and the staffs of several North American herbaria, to be permitted to examine a considerable proportion of the specimens of filamentous Myxophyceae upon which previous records in the literature have been based. Among other important collections, I have had access to that (in the form of fragments taken from the specimens in the British Museum and transmitted, with copies of the herbarium labels, by Mr. Tandy) made by J. W. H. Trail in Matto Grosso, Pará, and Amazonas in 1874-1875 and to those of Alberto Löfgren from São Paulo. The Trail collection constitutes the largest of the early accumulations of freshwater algae from Brazil; it is yet the most extensive collection of these plants from the Amazon basin. Dickie, in

Jour. Linn. Soc. Bot. 18: 123-132 (1880), catalogued this material in terminology and nomenclature scarcely comprehensible to us today; until the present time, not more than a half dozen of the Trail specimens of Myxophyceae has been re-interpreted in the light of modern knowledge of classification and morphology. Additional samples of blue-green algae secured in 1935-1937 in the states of São Paulo, Ceará, and Pará have recently been received from Dr. Stillmen Wright. All of this material, with that of my own collecting, is reviewed in the present article.

Specimens cited in the lists below are to be found in herbaria indicated by means of the following abbreviations: Ber, Botanisches Museum, Berlin-Dahlem; BM, British Museum (Natural History), London; D, my personal herbarium; F, Farlow Herbarium of Harvard University; L, Rijksherbarium, Leiden; Mi, Herbarium of the University of Michigan; Mo, Missouri Botanical Garden; N, New York Botanical Garden; O, Botaniske Museum, Oslo; R, Museu Nacional, Rio de Janeiro; S, Naturhistoriska Riksmuseet, Stockholm; T, Herbarium of Wm. Randolph Taylor; U, United States National Herbarium; Y, Herbarium of Yale University. I wish to express my gratitude to the members of the staffs of these and other herbaria for their cooperation during this study. I take pleasure in calling the reader's attention to the long list of persons in the first paper of this series (see above) who have given me assistance

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in many ways. I am indebted to Professor Wm. Randolph Taylor for offering suggestions concerning this manuscript. Much of the work was made possible through the aid of the Theresa Seessel Research Fellowship at Yale University.

THE STIGONEMATACEAE

I have not had the opportunity to examine the specimens upon which were based the following records of species published before the point of departure for nomenclature in the group (1887): *Stigonema compactum*, *S. compactum* var. *brasilense*, *S. mammosum*, and *Hapalosiphon fuscescens* of Wille, Bih. t. k. Sv. Vet.-Akad. Handl. 8(18): 5 (1884).

Capsosira Brebissonii Kütz. ex Born. & Flah., Ann. Sci. Nat. VII Bot. 5: 79 (1887). *Stigonema compactum* Gardn.,² Univ. California Publ. Bot. 14: 10, pl. 1, f. 9 (1927) [not Kirchn., not Borzi, not Wille]. *S. hormoides* var. *compactum* Geitler, Rabenh. Kryptogamen-Fl. 14: 500 (1931). *S. Gardneri* J. DeToni, Noterelle Nomencl. Algol. III (1936).—Here are placed the specimens which Dickie, Jour. Linn. Soc. Bot. 18: 126 (1880), reported under the names *Inactis obscura* (in part, not as to type) and *I. fasciculata* [see Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937)]. Material seen: AMAZONAS: dripping sandstone at Cachoeira de Tarumã, Rio Negro near Manáos, *Trail*, *Trail* 73, 31 July 1874 (BM); behind the fall, Cachoeira Grande, Manáos, *Trail*, 27 Dec. 1874 (BM).

STIGONEMA HORMOIDES (Kütz.) Born. & Flah., *ibid.* 5: 68 (1887). *Sommierella hormoides* Borzi, N. Giorn. Bot. Ital., N. S. 24: 79 (1917).—One collection seen: PARÁ: with *S. ocellatum* on weeds in reservoir 1 km. northeast of Santa Izabel, Belém, *S. Wright* 1612, 10 July 1935 (D, F, L, N, R).

STIGONEMA OCELLATUM (Dillw.) Thur. ex Born. & Flah., *ibid.* 5: 69 (1887); Möbius, *Hedwigia* 34: 159 (1895); Borge, Ark. f. Bot. 15(13): 94 (1919). *S. ocellatum* var. *Braunii* Hieron., *Hedwigia* 34: 159 (1895); Borge, loc. cit. (1919). *S. anomalum* Blanchard, *Rhodora* 15: 194 (1913).—This species was reported from Minas Geraes by Hariot, *Notarisia* 6: 1217 (1891). Specimens seen: SÃO PAULO: in Sümpfen, Itajahy, Serra d'Itatiara, *E. Ule* 15, Feb. 1894 (Ber); *Löfgren* 749, 762 (S); with *Hapalosiphon pumilus*, *Löfgren* 178 (S), 748 (F). PARÁ: Belém: in reservoir 1 km. northeast of Santa Izabel, *Wright* 1611, 1612, 10 July 1935 (D, F, L, N, R).

STIGONEMA PANNIFORME (Ag.) Born. & Flah., *S. MINUTUM* (Ag.) Hass. ex Born. & Flah. & *S. turfaceum* (Engl. Bot.) Cooke ex Born. & Flah. are reported from Minas Geraes and Amazonas by Hariot, *Notarisia* 6: 1217, 1218 (1891), and by Möbius, Ber. Deutsch. Bot. Ges. 10: 24 (1892). *S. GLAZIOVI* Henn. & Hieron., *Hedwigia* 34: 171 (1895), is described from material collected by Glaziou in Brazil.

²THE TYPE of *Stigonema compactum* Gardn. in the Farlow Herbarium, CHINA: Kushan near Foochow, Fukien Province, *H. H. Chung* A420, 1926, is similar to the specimen of *Capsosira Brebissonii* from Sweden in Wittrock & Nordstedt, Alg. exs. 183 (F), cited by Bornet & Flahault.

STIGONEMA ROBUSTUM Gardn., Univ. California Publ. Bot. 14: 9, pl. 4 (1927).—One specimen seen: SÃO PAULO: Serra de Bocaina, *Löfgren* 36, Apr. 1894 (S).

FISCHERELLA AMBIGUA (Born. & Flah.) Gom., Jour. de Bot. 9: 52 (1895). *Scytonema ambiguum* Kütz. ex Born. & Flah., *ibid.* 5: 100 (1887). *S. badium* Wille ex Born. & Flah. sp. inquir., *ibid.* 5: 111 (1887); Wille, Bull. Torrey Bot. Club 6: 184 (1877). *Phormidium interruptum* var. *rigidum* Gardn., Mem. New York Bot. Gard. 7: 44 (1927), sec. specim. typic. (N).—Bornet & Flahault (*ibid.*) report a specimen of this species from Brazil. The material seen here is that mentioned by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880), under the name *Hypheothrix olivacea* [see Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937)]: PARÁ: Igarapé do Bom Jardim, Rio Tapajós, *Trail* 71, Mar. 1874 (BM).

HAPALOSIPHON PUMILUS (Kütz.) Kirchn. ex Born. & Flah., Ann. Sci. Nat. VII Bot. 5: 61 (1887). *H. fontinalis* (Ag.) Born., Bull. Soc. Bot. France 36: 156 (1888). *H. brasiliensis* Borge, Ark. f. Bot. 15(13): 94, Taf. 7, f. 7 (1919).—I find no justification for separating the TYPE material of *H. brasiliensis* from specimens of *H. pumilus* cited by Bornet & Flahault. The author of the species omitted a description and figure of the creeping primary filaments in the specimens. Similar omissions have been made in the original and current descriptions of several other species of *Hapalosiphon* proposed since the appearance of Bornet & Flahault's 'Révision.' Only a careful comparative study of all the historical specimens involved, of course, will demonstrate the true status of these novelties. Specimens not examined here have been recorded as *H. pumilus* from Brazil by Bornet & Flahault (loc. cit.) and Borge (loc. cit.), from Minas Geraes by Möbius in *Hedwigia* 34: 179 (1895), and from Matto Grosso by Schmidle, *idem* 40: 51 (1901). Specimens seen: SÃO PAULO: på grasskån, São Paulo, *Löfgren* 748 (TYPE of *H. brasiliensis*, S; isotype, F); *Löfgren* 178 (S); no reservatório Guarapiranga perto de Santa Amaro, *Wright* 4912, 18 Oct. 1935 (D, L). CEARÁ: Fortaleza: shallow backwaters of açude, Mondubim, *Drouet* 1466, 22 Oct. 1935 (D); on larger plants in stream, Barra do Ceará, *Drouet* 1433, 26 Sept. 1935 (BM, D, F, M, N, R, Y). Quixadá: on grasses, Açude Boa Água, *Drouet* 1538, 2 Sept. 1935 (BM, D, F, L, N, R). MARANHÃO: shallow waters of reservoir, Sacavém, Ilha de São Luiz, *Drouet* 1317, 1322, 24 July 1935 (D, L, N).

HAPALOSIPHON BARONII West & West f. is reported from Matto Grosso by Schmidle, *Hedwigia* 40: 51 (1901).

LOEFGRENIA ANOMALA Gom. in Wittrock & Nordstedt, Alg. exs. 28: 1350 (1896); Edwall, Bol. Comm. geogr. & geol. S. Paulo 11: 187 (1896); Gomont, Bot. Notiser 1897: 90 (1897); Borge, Ark. f. Bot. 15(13): 95 (1919); Geitler, Rabenh. Kryptogamen-Fl. 14: 472 (1931).—The material cited here from PARÁ is neither very well developed nor admirably preserved; I place it here with hesitation. Specimens seen: SÃO

PAULO: ad Santa Amaro Batrachosperma aliasque plantas submersas investiens, *Löfgren* (isotype of *L. anomala*, Wittr. & Nordst., Alg. exs. 1350, N). PARÁ: on woodwork of mill-race at reservoir, Santa Izabel, Belém, *Drouet* 1535, 10 July 1935 (BM, D, F, Geitler, N).

NOTOSTOCHOPSIS LOBATUS Wood ex Born. & Flah., *ibid.*, 5: 80 (1887); Wittrock & Nordstedt, Alg. exs. 12: 578 (1883); Edwail, Bol. Comm. geogr. & geol. S. Paulo 11: 188 (1896); Borge, Ark. f. Bot. 15(13): 94 (1919). *Mazaea rivularioides* Born. & Grun. ex Born. & Flah. *pro synon.*, loc. cit. (1887); Bornet & Grunow, Bull. Soc. Bot. France 28: 287, pl. 7 (1881); Piuggari (as '*Borzia rivularioides* Grunow & Borrel')—see *Drouet*, Amer. Jour. Bot. 24: 600, footnote 4. 1937, Anal. Soc. cient. Argentina 11: 213 (1881). *Nostochopsis Hansgirgii* var. *sphaericus* Gardn.³ Univ. California Publ. Bot. 14: 11, pl. 1, f. 10 (1927).—This species has been reported from the states of Rio de Janeiro and São Paulo by Wille, Bih. t. k. Sv. Vet.-Akad. Handl. 8(18): 7 (1884), and by Möbius, Ber. Deutsch. Bot. Ges. 10: 24 (1892). Specimens seen: SÃO PAULO: '*Mazaea rivularioides*, Brésil,' ex herb. Bornet (F); Iguaçu, Iporanga, Piuggari, Juli 1873 (isotype of *Mazaea rivularioides*, Ber); ad palos et ligna aquae rapide fluentis ad Fazenda Bom Fim do Snr. João Manoel d'Almeida Barboza prope Campinas, *Löfgren* 201, 15 Aug. 1882 (Os; Wittr. & Nordst., Alg. exs. 578, D, N, U).

THE RIVULARIACEAE

AMPHITHRIX JANTHINA (Mont.) Born. & Flah., Ann. Sci. Nat. VII Bot. 3: 344 (1886).—One collection, CEARÁ: Fortaleza: on concrete-work, outlet of large lake, *Drouet* 1335, 28 July 1935 (D, F, Mi, N, R).

CALOTHRIX JULIANA (Menegh.) Born. & Flah., *ibid.* 3: 348 (1886).—The one specimen, with sheaths thin but brownish, is the basis for the record of *Lyngbya rufescens* by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880) [see *Drouet*, Amer. Jour. Bot. 24: 600, footnote 4 (1937)]: AMAZONAS: on stone from edge of water at Labréa, Rio Purús, *Trail* 92, 1 Oct. 1874 (BM).

CALOTHRIX AERUGINEA (Kütz.) Thur. ex Born. & Flah. is reported from Brazil by Möbius, Notarista 5: 1070 (1890).

CALOTHRIX PILOSA Harv. ex Born. & Flah., *ibid.* 3: 363 (1886); Harvey, Nereis Boreali-Americana 3: 106 (1857). *C. dura* Harv. ex Born. & Flah. *pro synon.*, loc. cit. (1886); Harvey, *ibid.* 3: 107 (1857). *Tildenia pilosa* Poljansky, Bull. Jard. Bot. Princ. URSS 27: 327 (1928). *T. dura* Poljansky, *ibid.* 27: 329 (1928).

³ THE TYPE of *N. Hansgirgii* var. *sphaericus* Gardn. in the Farlow Herbarium, CHINA: Kushan near Poochow, Fukien Province, *H. H. Chung* 4340, is indistinguishable from masses of small plants of *N. lobatus* in specimens cited by Bornet & Flahault. The tenuous filaments described by Gardner are conspicuously abnormal ones, with the included trichomes much shrunken. Unshrunken filaments and trichomes in the plants are similar to those of *N. lobatus*.

Setchelliella pilosa J. DeToni, Noterelle Nomencl. Algol. VIII (1936). *S. dura* J. DeToni, loc. cit. (1936).—Duplicate material of the original collections of *Calothrix pilosa* and *C. dura* from Key West by W. H. Harvey is on file in the Farlow Herbarium. The two specimens labeled *C. pilosa* are almost pure masses of the alga described under this name by Bornet & Flahault. The one bearing the name *C. dura* (in Harvey's handwriting) is a mixture of three species: *C. pilosa* (with filaments the counterparts of those in the specimens labeled *C. pilosa*), *Gardnerella corymbosa* (Grun.) J. DeToni [*Polythrix corymbosa* Grun. apud Born. & Flah.], and a species of *Dichothrix* rather similar to *D. penicillata* Born. & Flah. It is possible, since the last species predominates in certain parts of the mass, that Harvey referred to this form of *Dichothrix* as *Calothrix dura*; on the other hand, his rather uninformative description and figure may apply equally well to the *C. pilosa* present. Since the mass is composed of several species, including *C. pilosa*, we have no grounds to question Bornet & Flahault's disposal of the name *C. dura* as a synonym of *C. pilosa*.

Poljansky (*ibid.* 319 ff.) has discussed at length the status of Harvey's *C. pilosa* and *C. dura*. His concept of the former species has basis in two cited specimens: an apparently authentic specimen collected by W. H. Harvey at Key West in 1858 (!) and a specimen distributed as *C. pilosa* in Phycotheca Boreali-Americana 1167 (PUERTO RICO: on rocks, M. A. Howe, May 1903). *Calothrix dura*, on the other hand, is reinterpreted and redescribed by this author, not upon the basis of authentic material from Harvey or Bornet & Flahault, but upon a specimen collected half a century later at a station over 8000 km. distant from the type locality and on the shore of another ocean: the material distributed as *C. pilosa* in Phyc. Bor.-Amer. 859 (CALIFORNIA: on the bottoms of small pools near Point Carmel, Monterey County, W. A. Setchell, 10 June 1901). The two species were then placed, as indicated in the synonym above, in the ill-characterized genus *Tildenia* Kossinsk. [not Miq.]. With an equally small number of specimens at my disposal, I myself in a former paper, Univ. So. California Publ. Hancock Pacific Exped. 3: 21 (1936), came to just as unwarranted and naïve conclusions concerning the two specimens from the Phycotheca Boreali-Americana mentioned above. Then as now, I considered Phyc. Bor.-Amer. 1167 (Mo, N, Y) to be typical *Calothrix pilosa* Harv. ex Born. & Flah. But I suggested at that time that Phyc. Bor.-Amer. 859 (Mo) might more properly be disposed of under the name *Dichothrix seriata* Setch. & Gardn.

In the present study I have had the opportunity to examine carefully a large series of specimens of the marine species of *Calothrix*, including many of those cited in Bornet & Flahault's 'Révision.' Among others were the specimens treated in my discussion of the Hancock Expedition Myxophyceae (see above). It is now evident that the material in Phyc. Bor.-Amer. 859 (Mo, N, Y) is not to be considered a mem-

ber of the genus *Dichothrix* but rather as a large form of *Calothrix crustacea* Thur. ex Born. & Flah. collected and preserved at a season when the masses of hormogonia were in active development. Here the trichome of almost every filament has broken up into short segments, such hormogonia in many cases consisting of a single cell. Upon beginning development, each hormogonium has grown into a short filament characteristic of *C. crustacea* as represented in the specimens of the monographers. Mutual pressure of cells and young trichomes upon each other has caused ruptures of the sheaths, and almost every conceivable type of 'false branching' of the old filaments is abundant and conspicuous throughout the mass. Similar monstrosities and anomalous developmental phenomena (i.e., such as are not described in detail in diagnoses of species as written by the monographers, but are curiously explained by certain Russian myxophylobes by means of the phrases 'accélération phylogénétique,' 'phases prophétiques,' 'précession des caractères,' etc.) may be demonstrated in most of the thick-sheathed species of *Calothrix* wherein hormogonial masses are beginning development. They are encountered commonly in *C. pulvinata* Born. & Flah. and *C. parietina* Born. & Flah. under such conditions. In *C. vivipara* Born. & Flah. and a few related species, however, such branchings are common and abundant in masses in which hormogonia are infrequent. The material described and distributed under the name *Scytonema fuliginosum* Tilden, Amer. Alg. 629 [TERRITORY OF HAWAII: beach, Pakala Plantation, Hawaii, J. E. Tilden, 4 July 1900 (D, Mo)] should likewise be placed in the species *Calothrix crustacea*.⁴ Upon the basis of this collection, Kossinskaja (see footnote 4 of this paper) founded the genus *Tildenia*, the chief distinctive character of which is the lack of hairs at the apices of most of the trichomes. Careful examination of the original material show that most of the trichomes do possess hairs at their apices, and all show at least traces and remnants of them. Here during the formation of hormogonia the hairs have broken off and disappeared or have disintegrated in situ. The dead hair-cells or their disintegrating remains may be seen in the otherwise empty upper portions of the sheaths of many filaments. As in Phyc. Bor.-Amer. 859, multiple and simple false branching is abundant, and the young and recently developed filaments are those characteristic of *C. crustacea*. Heterocysts are present at the bases of all the young filaments; the older filaments are so long that they break during the process of being mounted on the slide, and it is difficult to find heterocysts at the bases of such broken filaments.

One collection of *C. pilosa* from Brazil: RIO GRANDE DO NORTE: on rocks in and about tide pools along

beach south of Natal, Drouet 1527, 19 June 1935 (D, F, L, N, R, S).

CALOTHRIX PARIETINA (Näg.) Thur. ex Born. & Flah., *ibid.* 3: 366 (1886). *Mastigothrix turgida* Wolle ex Forti, Syll. Myxophyc. 632 (1907); Wolle, Bull. Torrey Bot. Club 6: 184 (1877).—One collection: ALAGÓAS: base of wall of Palácio Hotel, Maceió, Drouet 1261, 16 June 1935 (D, L, N).

CALOTHRIX BRAUNII Born. & Flah., *ibid.* 3: 363 (1886). *Inactis obscura* Dickie ex Gom. sp. excl., *idem.* 15: 329 (1892); Dickie, Jour. Linn. Soc. Bot. 18: 126 (1880); Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937).—Three specimens in Dickie's herbarium are marked as *Inactis obscura*. The one cited here, which I take to be the type as Dickie himself would have indicated, is further annotated 'n. sp.' The filaments of this material match the original description of *I. obscura* better than do those of the other two specimens concerned. One of the latter, also from the Cachoeira Grande at Manáos, is treated in this paper under *Capsosira Brebissonii*; the other, from the Cachoeira de Tarumá near Manáos, is a mass of *Entophysalis* sp. The last specimen is probably the one which Gomont examined when he explained that *Inactis obscura* Dickie should be placed in the genus *Entophysalis*. One specimen of *Calothrix Braunii*: AMAZONAS: on soft sandstone from beneath the fall, Cachoeira Grande, Manáos, Trail, 27 Dec. 1874 (TYPE of *Inactis obscura*, BM).

RAPHIDIOPSIS CURVATA Fritsch & Rich, Trans. Roy. Soc. South Africa 18: 91, f. 32 (1929).—The Brazilian specimens listed here appear to find a proper place in this species; I have not had the opportunity, however, to compare this material with the original South African collections. The form is abundantly represented in the plankton of lakes and more or less permanent pools of fresh water in northeastern Brazil. From the appearance of the samples, it may occur in such quantities as to form water-blooms. The trichomes are evaginate and needle-like, very sharply pointed at either end. They are rigid and easily broken, usually curved or spiraled, not seldom straight or slightly curved. In diameter of the thickest portions they range in these collections from 1.5 μ to 4.5 μ . Typically unconstricted at the cross-walls, they rarely are conspicuously torulose. The cells are quadrate or longer than broad, in a few cases shorter than broad. The attenuation at one or both ends of the trichome may involve one or several cells. Reproduction is by fragmentation (formation of hormogonia) and by the development of thick-walled resting spores from cells in the broad portions of the trichomes. The spores in our material are similar to those described by Fritsch & Rich: the mature (thick-walled) spores are somewhat broader than the adjacent vegetative cells and 1½–3 times as long as broad; within the wall, the protoplasm is homogeneous and hyaline except for a few large refringent granules. The vegetative cells all contain pseudovacuoles or bear indications of having at one time possessed them. The pointed apices are often without large pseudovacuoles, even though it is evident

⁴ The synonymy of this species may be presented thus: *CALOTHRIX CRUSTACEA* Thur. ex Born. & Flah., Ann. Sci. Nat. VII Bot. 3: 359 (1886). *Scytonema fuliginosum* Tilden, Amer. Alg. 7: 629 (1909). *Tildenia fuliginosa* Kossinskaja, Not. Syst. Inst. Crypt. Hort. Bot. Princip. URSS 4: 77 (1926). *T. fuliginosa* var. *symmetrica* Kossinskaja, *ibid.* 79 (1926). *Setchelliaella fuliginosa* J. DeToni, Noterelle Nomencl. Algol. VIII (1936).

that at one time during the life-history of the trichome they possessed such bodies. It is apparently such apices as these which Fritsch & Rich describe as being "mucilaginous" and "a solid gelatinous bristle." In one sample, Wright 2022 cited below, many trichomes have at one end a hyaline refringent body which resembles a heterocyst. This body appears to be a narrowly conical cell, the base of which is attached to a vegetative cell. If it is a true heterocyst—and more satisfactory material than that at hand will be necessary to prove this point beyond doubt—the affinities of the genus with the Rivulariaceae should become evident. A number of collections lack spores; it is probable that as in *Aphanizomenon flos-aquae* Born. & Flah. and other heterocystous planktonic species which contain pseudovacuoles spores and heterocysts are formed only during certain restricted phases in the developmental history of the mass. Specimens seen: PARAÍHYBA: Mojeiro Baixo: Açude Linda Flor, Wright 2028, 16 Apr. 1934 (D, N); Açude Mendonça, Wright 2022, 29 Mar. 1934 (BM, D, Mi, N, R). Campina Grande: lake, Wright 1573, 28 Nov. 1934 (D, N); Açude Lapa, Wright 1559, 17 Jan. 1935 (D, N); Açude Simão, Wright 1967, 1999, 28 Feb. 1934 (D, N), 1569, 1582, 14 Aug. 1934 (BM, D, N, R), 1572, 19 Jan. 1935 (D, N), 1603, 2 Mar. 1935 (D, N). CEARÁ: Fortaleza: roadside pool near Siqueira, near Porangaba, S. Wright, 30 Oct. 1937 (BM, D, F, Mi, N, R).

DICHOTRIX OLIVACEA (Hook.) Born. & Flah., Ann. Sci. Nat. VII Bot. 3: 375 (1886). *Lyngbya noronhae* Dickie ex Forti, Syll. Myxophyc. 290 (1907); Dickie, Jour. Linn. Soc. Bot. 14: 365 (1874); Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937).—One specimen, not well preserved and with much-shrunken trichomes: FERNANDO DE NORONHA: Platform Island, H. Moseley, Challenger Expedition, Bottle 30 (TYPE of *L. noronhae*, BM).

GLOBOTRICHIA PISUM (Ag.) Thur. ex Born. & Flah., ibid. 4: 366 (1886); Wittröck & Nordstedt, Alg. exs. 14: 660c (1884); Borge, Ark. f. Bot. 15(13): 95 (1919). *Rivularia Pisum* Ag. ex Born. & Flah. *pro synon.*, loc. cit. (1886). *R. paradoxa* Forti, Syll. Myxophyc. 672 (1907). *Zonotrichia paradoxa* Wolle ex Forti *pro synon.*, ibid. 636, 672 (1907); Wolle, Bull. Torrey Bot. Club 6: 138 (1877).—Reported from Brazil by Bornet & Flahault (ibid.) and from Matto Grosso by Schmidle, Hedwigia 40: 51 (1901). One specimen seen: SÃO PAULO: ad Tanque Grande de Bom Fim, São Paulo, Löfgren 206, 10 Sept. 1882 (Wittr. & Nordst., Alg. exs. 660c, N).

GLOBOTRICHIA NATANS (Hedw.) Rabenh. ex Born. & Flah., ibid. 4: 369 (1886); Borge, Ark. f. Bot. 15(13): 95 (1919). *Rivularia natans* Welm. ex Born. & Flah. *pro synon.*, loc. cit. (1886). *Calothrix scytoneimicola* var. *brasilensis* Borge, Ark. f. Bot. 19(17): 5, Taf. 1, f. 20 (1925). *Rivularia* (*Gloeotrichia*) *flagelliformis* Gardn., Mem. New York Bot. Gard. 7: 71 (1927).—The TYPE material of *Calothrix scytoneimicola* var. *brasilensis* is preserved in liquid; it contains characteristic young plants of *Gloeotrichia natans*

attached to bits of *Utricularia*. Many of the plants have become dissociated, however, so that the individual trichomes lie free in the preservative. The trichomes thus free of the surrounding jelly appear to be the basis for Borge's description of this variety, as the published figure would indicate. A few immature spores are present. Specimens seen: SÃO PAULO: A. Löfgren, 29 May 1896 (F, N); Löfgren 219 (S). Matto Grosso: under *Utricularia oligosperma*, São Luiz de Cáceres, F. C. Hoehne 52, 9 Jan. 1914 (TYPE of *Calothrix scytoneimicola* var. *brasilensis*, S). PERNAMBUCO: Rio São Francisco, Jatobá, Wright 2045, 21 Sept. 1933 (D, F, N, R); with *Anabaena Fuellebornii*, Açude da Nação, Villa Bella, Wright 2021, 11 June 1934 (BM, D, F, Mi, N, R, Y). PARAÍHYBA: pond near Campina Grande, Wright 2013, July 1934 (D). CEARÁ: Fortaleza: in temporary reservoir in Rio Maranguapinho, Barro Vermelho, Drouet 1498, 22 Nov. 1935 (BM, D, F, Mi, N, R, Y).

GLOBOTRICHIA LONGICAUDA Schmidle and G. PILGEBI Schmidle, Hedwigia 40: 51, 52 (1901), are described from material collected in Matto Grosso.

THE NOSTOCACEAE

To be referred questionably to this family is the record of *Nostoc laevigatum* by Martens, Vidensk. Medd. Dansk naturh. Foren. 22: 298 (1870), material of which I have not seen. The original specimens upon which Dickie, Jour. Linn. Soc. Bot. 18: 126 (1880), based his Brazilian reports of *Sphaerozyga oscillarioides* and *Anabaena bulbosa* prove to be sporeless or otherwise indeterminate masses of species of Nostocaceae. Dickie's record (loc. cit.) of *Anabaena gigantea* is excluded from this treatment: the authentic specimens labeled thus in the British Museum should be placed in the desmidaceous genus *Hyalotheca*.

NOSTOC HEDERULAE Menegh. ex Born. & Flah. is reported from the city of Rio de Janeiro by Möbius, Hedwigia 28: 313 (1889).

NOSTOC CARNEUM Ag. ex Born. & Flah., Ann. Sci. Nat. VII Bot. 7: 196 (1888); Wittröck & Nordstedt, Alg. exs. 32: 1512 (1903); Borge, in part, Ark. f. Bot. 15(13): 93 (1919).—One collection seen: SÃO PAULO: ad rotam serratarinae, Serra da Cantareira, Löfgren 643, 27 Aug. 1894 (S; Wittr. & Nordst., Alg. exs. 1512, N).

NOSTOC PISCINALE Kütz. ex Born. & Flah., ibid. 7: 194 (1888); Wittröck & Nordstedt, Alg. exs. 8: 398 (1880); Edwall, Bol. Comm. geogr. & geol. S. Paulo 11: 188 (1896); Borge, Ark. f. Bot. 15(13): 92 (1919).—The specimen cited here from Matto Grosso is one of several recorded by Dickie, Jour. Linn. Soc. Bot. 18: 126 (1880), as *Cylindrospermum riparium*. Collections seen: SÃO PAULO: in fossa ad Pasto de Olaria prope Pirassununga, Löfgren 115, 18 Jan. 1880 (S; Wittr. & Nordst., Alg. exs. 398, N); Löfgren 138 (S). MATTO GROSSO: on mud from stagnant pool at Santo Antonio, lower falls of Rio Madeira, Trail, 27 May 1874 (BM). CEARÁ: on other algae in pools in Rio Pacoty 6 km. south of Aquiraz, Drouet 1453, 15 Oct. 1935 (D, N). AMAZONAS: from surface of a puddle

at Estaleiro, east bank of Rio Madeira, *Trail* 35, 18 May 1874 (BM).

NOSTOC SPONGIAEFORME Ag. ex Born. & Flah., *ibid.* 7: 197 (1888).—The specimen ascribed here to São Paulo was reported by Borge, *Ark. f. Bot.* 15(13): 93 (1919), under the name *N. carneum*. Material seen: SÃO PAULO: *Löfgren* 647 (F, S). *PARAHYBA*: Açude Alto, Pomhal, *Wright* 2006, 2023, 23 June 1934 (BM, D, F, L, Mi, N, R, Y).

NOSTOC PASSERINIANUM (Not.) Born. & Thur. ex Born. & Flah., *ibid.* 7: 199 (1888).—I place here the specimen recorded by Borge, *Ark. f. Bot.* 15(13): 93 (1919), as *N. Muscorum*: SÃO PAULO: Serra da Bocaina, *Löfgren* 51, Apr. 1894 (S).

NOSTOC MUSCORUM Ag. ex Born. & Flah. is reported from São Paulo by Möbius, *Hedwigia* 34: 179 (1895).

NOSTOC COMMUNE Vauch. ex Born. & Flah., *ibid.* 7: 203 (1888).—The not-too-well developed plants cited here were recorded under the name *N. laevigatum* by Dickie, *Jour. Linn. Soc. Bot.* 18: 125 (1880): PARÁ: moist sandstone cliff beside the Amazon, Obidos, *Trail* 129, 5 Feb. 1874 (BM). AMAZONAS: on dripping cliff, Barreiras de Maniwa, Rio Purús, *Trail* 111, 29 Sept. 1874 (BM).

ANABAENA VARIABILIS Kütz. ex Born. & Flah. is reported from marine [??] waters at Cabo Frio, State of Rio de Janeiro, by Möbius, *Hedwigia* 28: 313 (1889).

ANABAENA SPHAERICA Born. & Flah., *Ann. Sci. Nat. VII Bot.* 7: 228 (1888).—CEARÁ: Aquiraz: with *Nostoc piscinale*, Rio Pacoty 6 km. south of Aquiraz, *Drouet* 1453, 15 Oct. 1935 (D, N). Maranguape: with *Tolypothrix distorta*, Açude Bom Sucesso, S. Wright, 4 Nov. 1937 (D, F, N, R); Açude Trapiá, *Drouet* 1491, 1 Nov. 1935 (BM, D, F, L, Mi, N, R, Y).

ANABAENA FLOS-AQUAE (Lyngb.) Bréb. ex Born. & Flah., *ibid.* 7: 228 (1888).—The material cited here has no spores; it comprises a considerable part of the plankton of two lakes: PARAHYBA: Açude Soledade, Soledade, *Wright* 1575, 1 Dec. 1934 (D, L, N); lake near Campina Grande, *Wright* 1573, 28 Nov. 1934 (D).

ANABAENA SPIROIDES Klebahn, *Flora* 80: 268, Taf. IV, f. 11-13 (1895).—The spores in the one collection placed here are spherical or ovoid-spherical in the mature (thick-walled) condition. The plants may prove to be a form of *A. flos-aquae*. CEARÁ: Maranguape: Açude Frexeiras, S. Wright, 11 Sept. 1937 (D, F, N, R).

ANABAENA CIRCINALIS (Kütz.) Rabenh. ex Born. & Flah., *ibid.* 7: 230 (1888). *Microcystis lobata* Dickie, *Journ. Linn. Soc. Bot.* 18: 128 (1880). *Anabaena scabra* Dickie ex Born. & Flah. *sp. inquir.*, *ibid.* 7: 239 (1888); Dickie, *ibid.* 18: 128 (1880).—THE TYPES of *A. scabra* and *Microcystis lobata* are easily recognizable preparations of *A. circinalis*. Specimens seen: PARAHYBA: Açude Laranjeiro near Campina Grande, *Wright* 1984 (sporis nullis), 10 Nov. 1933 (D); Açude Simão near Campina Grande, *Wright* 1967, 1999 (sporis nullis), 28 Feb. 1934 (D, L, N, R).

PARÁ: green layer on top of water of Rio Tapajóz, *Trail* 74 (TYPE of *Microcystis lobata*, BM); from Rio Tapajóz at Santarém, *Trail* 56, 17 Feb. 1875 (TYPE of *Anabaena scabra*, BM).

ANABAENA INAEQUALIS (Kütz.) Born. & Flah., *ibid.* 7: 231 (1888). *A. Kuetzingiana* J. DeToni, *Noterelle Nomencl. Algol. VIII* (1936).—One collection, CEARÁ: Pacatuba: in Rio Pacoty near Fortaleza-Reife Road, *Drouet* 1502, 24 Nov. 1935 (BM, D, F, L, N, R, Y).

ANABAENA FUELLEBORNI Schmidle, *Engl. Bot. Jahrb.* 32: 61, Taf. 1, f. 4 (1902).—The material cited here is similar to that figured and described by Schmidle; I place it here even though I have not had the opportunity to examine the original specimens from Africa. PERNAMBUCO: Açude da Nação, Villa Bella, *Wright* 2021, 11 June 1934 (BM, D, F, Mi, N, R, Y).

ANABAENA OSCILLARIOIDES Bory ex Born. & Flah., *ibid.* 7: 233 (1888).—Schmidle, *Hedwigia* 40: 53 (1901), has recorded this species from Matto Grosso. One collection seen: CEARÁ: in pools in Rio Pacoty 6 km. south of Aquiraz, *Drouet* 1454, 15 Oct. 1935 (D, F, L, N, R).

RICHELIA INTRACELLULARIS Schmidt is recorded from the coast of Santa Catharina by Marques da Cunha & Fonseca, *Mem. Inst. Oswaldo Cruz* 10: 100 (1918), and by Lutz, Souza Araújo & Fonseca Filho, *idem* 10: 160 (1918).

CYLINDROSPERMUM STAGNALE (Kütz.) Born. & Flah., *ibid.* 7: 250 (1888).—One of the specimens recorded by Dickie, *Jour. Linn. Soc. Bot.* 18: 126 (1880), as *C. caeruleum* (not the TYPE) should be placed here: AMAZONAS: from surface of moist clayey sand, Barreiras de Hyutanaham, Rio Purús, *Trail*, 28 Sept. 1874 (BM).

CYLINDROSPERMUM MAJUS Kütz. ex Born. & Flah., *ibid.* 7: 252 (1888). *C. janthinum* Dickie ex Born. & Flah. *sp. inquir.*, *ibid.* 7: 255 (1888); Dickie, *Jour. Linn. Soc. Bot.* 18: 126 (1880).—THE TYPE of *C. janthinum* is a mixture of two species: *C. muscicola*, represented entirely by old and much shriveled spores, and *C. majus*, in an actively growing state and with few almost mature spores. From Dickie's description, the latter component is to be designated by the name *C. janthinum*. To *C. majus* also I ascribe here the specimens recorded by Dickie, *ibid.* 18: 126, 127 (1880), as *Hypheothrix thermalis* [see *Drouet*, *Amer. Jour. Bot.* 24: 600, footnote 4 (1937)] and *CylindrospERMUM RIPARIUM*. Bornet & Flahault (*ibid.*) report a specimen of *C. majus* collected by Piaggari in Brazil. Specimens seen: AMAZONAS: moist earth in forest at Porto Salvo, Rio Purús, *Trail*, 3 Oct. 1874 (TYPE of *C. janthinum*, BM); moist soil, overhanging clay rock, and dripping rock, Barreiras de Hyutanaham, Rio Purús, 65° 39' W. Long., *Trail*, *Trail* 2, 79, 28 Sept. 1874 (BM).

CYLINDROSPERMUM LICHENIFORME (Bory) Kütz. ex Born. & Flah., *ibid.* 7: 253 (1888); Wittrock & Nordstedt, *Alg. exs.* 14: 680 (1884); Borge, *Ark. f. Bot.* 15(13): 93 (1919).—One collection seen: SÃO PAULO:

in flumine Tamanduatehy prope São Paulo, *Löfgren* 175, 1 Mar. 1882 (Witt. & Nordst., Alg. exs. 680, N).

CYLINDROSPERMUM MUSCICOLA Kütz. ex Born. & Flah., *ibid.* 7: 254 (1888). *C. caeruleum* Dickie ex Born. & Flah. *sp. inquir.*, *ibid.* 7: 255 (1888); Dickie, *Jour. Linn. Soc. Bot.* 18: 126 (1880).—Here is placed, along with the type of *C. caeruleum*, a part of the material reported by Dickie (loc. cit.) as *C. riparium*. Specimens seen: PERNAMBUCO: on mud and in pools, Laboratorio da Piscicultura, Recife, *Drouet* 1263, 17 June 1935 (D, F, L, N, R). AMAZONAS: on damp soil (moist earth) in forest, Porto Salvo, Rio Purús, *Trail*, *Trail* 45 (TYPE of *C. caeruleum*, BM), 101, 3 Oct. 1874 (BM).

CYLINDROSPERMUM CATENATUM Ralfs ex Born. & Flah. is reported from the city of Rio de Janeiro by Möbius, *Ber. Deutsch. Bot. Ges.* 10: 25 (1892).

AULOSIRA IMPLEXA Born. & Flah., *ibid.* 7: 257 (1888), *Bull. Soc. Bot. France* 32: 120, pl. 4, f. 4 (1885).—Our material has somewhat smaller measurements of filaments and cells than is seen in the type collection, URUGUAY: Montevideo, *J. Archavaleta*, Aug. 1884 (Witt. & Nordst., Alg. exs. 787, N). However, the measurements are similar in all details to those seen in the collection by Lagerheim from Ecuador in Witt. & Nordst., Alg. exs. 1323 (N). One Brazilian collection: CEARÁ: Fortaleza: in a freshwater pond by beach, Urubú, *Drouet* 1336, 1 Aug. 1935 (BM, D, F, Mi, N, R, Y).

THE SCYTONEMATACEAE

Modern nomenclature cannot be applied to the following records until the historic specimens have been reinterpreted: *Scytonema Panicéi* of Martens, *Vidensk. Medd. Dansk naturh. Foren.* 22: 298 (1870); *S. (Symphyosiphon) hirtulum* of Zeller, *idem* 28–30: 426 (1876); and *Scytonema immersum* of Wille, *Bih. t. k. Sv. Vet.-Akad. Handl.* 8(18): 5 (1884). The specimen upon which Dickie, *Jour. Linn. Soc. Bot.* 18: 127 (1880), based his report of *Beggiatoa arachnoidea* is indeterminable material of a species of *Scytonema*.

FREMYELLA TENERA (Born. & Flah.) J. DeToni, *Noterelle Nomencl. Algol.* VIII (1936). *Microchaete tenera* Thur. ex Born. & Flah., *Ann. Sci. Nat. VII Bot.* 5: 84 (1887).—Included here is the *M. tenera* var. *major* reported by Borge in *Ark. f. Bot.* 19(17): 5 (1925). Specimens seen: MATTO GROSSO: with *Gloeotrichia natans*, under *Utricularia oligosperma*, São Luiz de Cáceres, *F. C. Hoehne* 52, 9 Jan. 1914 (S). CEARÁ: Fortaleza: on Rhizoclonium in pools of fresh water in dunes, *Drouet* 1381, 24 Aug. 1935 (D).

TOLYPOTHRIX LANATA (Desv.) Wartm. ex Born. & Flah., *ibid.* 5: 120 (1887).—The material here cited has passed in the literature under the names *T. Aegropila* var. *bicolor* in Wittrock & Nordstedt, Alg. exs. 12: 580 (1883), and Edwall, *Bol. Comm. geogr. & geol. S. Paulo* 11: 189 (1896), and *T. tenuis* in Borge, *Ark. f. Bot.* 15(13): 93 (1919); São PAULO: in piscina ad Colonia Izabel prope Campinas, *Löfgren* 205, 5 Nov. 1882 (Witt. & Nordst., Alg. exs. 580, N, U).

TOLYPOTHRIX DISTORTA (Fl. Dan.) Kütz. ex Born. & Flah., *ibid.* 5: 119 (1887).—Specimens seen: CEARÁ: Açude Bom Sucesso near Maranguape, *S. Wright*, 4 Nov. 1937 (D, F, N, R); floating in Açude Forquilha, 18 km. east of Sobral, *S. Wright*, 26 Oct. 1937 (BM, D, F, Mi, N, R).

HASSALLIA BYSSOIDEA (Berk.) Hass. ex Born. & Flah., *ibid.* 5: 116 (1887). *Anabaena cypressaphila* Wolle ex Forti, *Syll. Myxophyc.* 456 (1907); Wolle, *F. W. Alg. U. S.* 1: 288 (1887). *Tolyptothrix byssoides* Kirchn. apud Forti, *ibid.* 561 (1907). *Scytonema (Petalonema) junipericola* Farlow, *Phyc. Bor.-Amer.* 16: 756 (1900).—The specimens cited here were distributed to various herbaria under the name *Scytonema Hofmannii*: MARANHÃO: on log, reservoir at Sacavém, Ilha de São Luiz, *Drouet* 1320, 24 July 1935 (D, F, N, R). PARÁ: on framework of shelter of fish tanks, Museu Paraense, Belém, *Drouet* 1288, 1 July 1935 (BM, D, F, L, Mi, N, R).

SCYTONEMA FIGURATUM Ag. ex Born. & Flah. [*S. mirabile* Born., not Wolle] is reported from Minas Geraes by Hariot, *Notarisia* 6: 1218 (1891), and from Rio de Janeiro by Möbius, *Ber. Deutsch. bot. Ges.* 10: 24 (1892).

SCYTONEMA TOLYPOTRICHOIDES Kütz. ex Born. & Flah., *Ann. Sci. Nat. VII Bot.* 5: 100 (1887). *S. gracile* var. *tolyptotrichoides* Witt. ex Born. & Flah. *sp. inquir.*, *ibid.* 5: 111 (1887); Wittrock apud Wolle, *Bull. Torrey Bot. Club* 8: 38 (1881).—It is with some hesitation that I cite and distribute the specimens below under this name. In many respects they resemble *S. figuratum* var. *Leprieurii* Born. & Flah.: CEARÁ: Quixadá: in a pond at fazenda Aroeiras, *Drouet* 1401, 2 Sept. 1935 (BM, D, F, Mi, N, R). Fortaleza: freshwater pool below dunes, Urubú, *Drouet* 1333, 24 Aug. 1935 (D).

SCYTONEMA HOFMANNII Ag. ex Born. & Flah., *ibid.* 5: 97 (1887).—I place under this name the specimens which Dickie, *Jour. Linn. Soc. Bot.* 18: 125, 127 (1880), recorded as *S. aerugineo-cinereum* and *Lyngbya guyanensis*.⁵ Other Brazilian specimens are reported as *Scytonema Hofmannii* from Minas Geraes and São Paulo by Hariot, *Notarisia* 6: 1218 (1891), and Edwall, *Bol. Comm. geogr. & geol. S. Paulo* 11: 189 (1896). Material seen: AMAZONAS: on damp rotting wood in igapó at Paricatuba, Rio Purús, *Trail*, 12 Oct. 1874 (BM); pieces of bark kept wet, Cuyabá or Linda Vista, Rio Purús, *Trail* 96, 13 Sept. 1874 (BM); Barreiras de Jutahí, Rio Solimões, *Trail* 47 (BM).

SCYTONEMA VARIUM Kütz. ex Born. & Flah., *ibid.* 5: 97 (1887); Möbius, *Ber. Deutsch. Bot. Ges.* 10: 25 (1892).—Here is placed the specimen labeled *S. Hofmannii* in Witt. & Nordst., Alg. exs. 16: 765c (1886), and listed under the same name by Borge,

⁵ By translating Dickie's report of *Lyngbya guyanensis* from Amazonas into the synonymy of Gomont's 'Monographie,' Forti in *Syll. Myxophyc.* 274 (1907) records the marine *L. semiplena* from this far inland region. See also Taylor, *Rév. Algol.* 3: 286 (1931), and Drouet, *Amer. Jour. Bot.* 24: 600, footnote 4 (1937).

Ark. f. Bot. 15(13): 93 (1919). Other material has been recorded as *S. varium* from Brazil by Bornet & Flahault (loc. cit.) and from Rio de Janeiro by Hariot, Notarisia 6: 1218 (1891). Specimens seen: DISTRICTO FEDERAL: auf Felsen, Tijuca, *Herb. Schwacke 863*, 1874 (Ber). S. S. PAULO: A. Löfgren (Witt. & Nordst., Alg. exs. 7656, N).

SCYTONEMA OCELLATUM Lyngb. ex Born. & Flah., 5: 95 (1887); Möbius, Ber. Deutsch. Bot. Ges. 10: 24 (1892).—A specimen is reported under this name from Brazil by Bornet & Flahault (ibid.). Material seen: DISTRICTO FEDERAL: auf Felsen, Corcovado, *Herb. Schwacke 863*, 1876 (Ber). PERNAMBUCO: on old wall below monastery, Olinda, *Drouet 1267*, 17 June 1935 (D). PARÁ: on moist rocks, Bosque Rodriguez Alves, Belém, *Drouet 1308*, 8 July 1935 (BM, D, F, L, Mi, N, R, Y).

SCYTONEMA JAVANICUM (Kütz.) Born. ex Born. & Flah., ibid. 5: 95 (1887). *S. hyalinum* Gardn.⁹ Univ. California Publ. Bot. 14: 7 (1927). *S. guyanense* var. *epiphyllum* Gardn.⁹ New York Acad. Sci. Sci. Surv. Porto Rico 8: 299 (1932).—Bornet & Flahault (loc. cit.) have reported material of *S. javanicum* from Brazil; Hariot, Notarisia 6: 1218 (1891), has recorded it from the city of Rio de Janeiro. The one collection seen was distributed to herbaria under the name *S. Hofmannii*: PARÁ: Belém: on wood floating in pool, Fonte Magnary, *Drouet 1536*, 17 July 1935 (D, L, N).

SCYTONEMA GUYANENSE (Mont.) Born. & Flah., ibid. 5: 94 (1887). *S. guyanense* var. *minus* Gardn., Mem. New York Bot. Gard. 7: 79 (1927).—Here I include the one specimen which Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880) [see Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937)], reported under the name *Oscillaria antillarum*. *Scytonema guyanense* is recorded for Brazil by Bornet & Flahault (ibid.) and for Minas Geraes by Hariot, Notarisia 6: 1218 (1891). Specimens seen: CEARÁ: Aquiraz: on open soil in high woods 3 km. northwest of Euzebio, *Drouet 1483*, 5 Nov. 1935 (D, F, L, Mi, N, R). AMAZONAS: on damp rotting tree in igapó at Pupunha, Rio Jurua, *Trail*, 5 Nov. 1874 (BM).

⁹In the many Chinese specimens in the Farlow Herbarium and in my personal herbarium designated by Gardner as *S. hyalinum*, I have found only plants with the characters of *S. javanicum* (as represented, however sparsely, in Witt. & Nordst., Alg. exs. 1510, N). The sheaths are all fairly thick, even in those filaments where the trichomes show no evident shrinking. The vacuolization of the protoplasm given as a distinctive character in the original description of *S. hyalinum* can have little value in a taxonomic sense, since vacuoles are produced in the protoplasm of most species of the Scytonemataceae under certain environmental conditions (such as those encountered during slow drying of the mass) and in several of the liquid preservatives. I am unable to detect the 'capitate apices' of the filaments which Gardner describes and figures. Among the many other specimens cited in the original description is the TYPE in the Farlow Herbarium: CHINA: Kushan near Foochow, Fukien Province, H. H. Chung 4387. The TYPE of *S. guyanense* var. *epiphyllum* in the Herbarium of the New York Botanical Garden, PUERTO RICO: on leaves of *Renalmia* sp., Las Marias, F. L. Stevens 1176, 10 July 1915, is a poorly developed mass of *S. javanicum*.

SCYTONEMA STUPOSUM (Kütz.) Born. ex Born. & Flah. is reported from Brazil by Bornet & Flahault, ibid. 5: 93 (1887).

SCYTONEMA COACTILE Mont. ex Born. & Flah., ibid. 5: 90 (1887). *S. coactile* var. *brasiliense* Nordst. ex Forti, Syll. Myxophyce. 501 (1907); Nordstedt in Wittrock & Nordstedt, Alg. exs. 10: 488 (1882), Bot. Notiser 1882: 58 (1882); Borge, Ark. f. Bot. 15(13): 93 (1919).—I can find no characters in the TYPE material of *S. coactile* var. *brasiliense* to distinguish it from specimens cited for the typical variety by Bornet & Flahault. Borge, ibid. 19(17): 5 (1925), has recorded a specimen under this varietal name from Matto Grosso. Specimens seen: S. S. PAULO: ad Olaria do Faustino prope Pirassununga, *Löfgren 162*, 21 Feb. 1880 (ISOTYPE of *S. coactile* var. *brasiliense*, Witt. & Nordst., Alg. exs. 488, N); *Löfgren 1506* (F). PARAHYBA: Açude Marinheiro near Campina Grande, *Wright 2035*, 9 Dec. 1933 (D).

SCYTONEMA CINCLINATUM (Kütz.) Thur. ex Born. & Flah. is reported from Brazil by Bornet & Flahault, ibid. 5: 89 (1887), from Matto Grosso by Schmidle, *Hedwigia* 40: 51 (1901), and from Amazonas by Möbius, Ber. Deutsch. Bot. Ges. 10: 24 (1892). *S. subtile* Möb. is recorded for São Paulo and Matto Grosso by Möbius, *Hedwigia* 34: 179 (1895), and by Schmidle (loc. cit.).

THE OSCILLATORIACEAE: ADDITIONAL NOTES

The following is a record of additional historical and hitherto unreported specimens which have come to my attention since the publication of my treatment of the Brazilian Oscillatoriaceae in Amer. Jour. Bot. 24: 600 ff. (1937). Those species appearing in the previous article are marked here with an asterisk (*). To be excluded from the family is the record of *Oscillaria americana* by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880); the specimen (BM) upon which this report was based is a member of the Chlorophyceae.

*PORPHYROSIPHON NOTARISII (Menegh.) Kütz. ex Gom.—The specimens listed here are those reported by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880), as *Lyngbya putealis*. See Drouet, Amer. Jour. Bot. 24: 604 (1937). Two specimens: PARÁ: small pool in pasture at Tonantins, upper Amazon, *Trail*, 24 Nov. 1874 (BM); fresh water below Monte Alegre, *Trail* (BM).

*PORPHYROSIPHON NOTARISII f. *amazonense*, forma nov. A forma typica vaginis hyalinis differt. TYPE in British Museum (Natural History): AMAZONAS: on damp ground in forest, Porto Salvo, Rio Purús, *Trail 133 bis*, 3 Oct. 1874.—Three specimens, only the TYPE bearing Trail's collection number, but all containing similar habitat and locality data, are placed here. Two of these prove to be the bases for the records of *Hypheothrix laminosa* and *Lyngbya arachnoidea* by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880) [see Drouet, Amer. Jour. Bot. 24: 604, footnote 4 (1937)]. All three specimens consist of bright green mats on soil, apparently taken from shaded situations in the forest. The filaments are similar to those of typical *Porphyrosiphon Notarisii*, except that here the red pigment is lacking in the sheaths. It is probable

that this absence of pigment is due to the lack of direct sunlight in the habitat; however, the herbarium labels do not enlighten us on this point.

SCHIZOTHRIX LAMYI Gom., Ann. Sci. Nat. VII Bot. 15: 323 (1892).—This is the material which Dickie (loc. cit.) recorded as *Hypheothrix vulpina* [see Drouet, loc. cit.]: AMAZONAS: dripping surface of clay cliff beside Rio Maues, *Trail 13*, 3 May 1874 (BM).

SCHIZOTHRIX LARDACEA (Ces.) Gom., ibid. 15: 307 (1892). *Leptothrix symplocoides* Dickie, Jour. Linn. Soc. Bot. 15: 240 (1876). *Hypheothrix lardacea* Hansg. in Dalla Torre & Sarnthein, Fl. Tirol, Verarlb. & Liechtenst. 2: 144 (1901).—One specimen, the basis of the record by Dickie, ibid. 18: 127 (1880), of *H. lateritia* var. *kermesina* [see Drouet, loc. cit.]: PARÁ: from bottom of a beer bottle stuck in garden wall, Belém, *Trail 166*, 6 Feb. 1875 (BM).

MICROCOLEUS CHTHONOPLASTES (Fl. Dan.) Thur. ex Gom., ibid. 15: 353 (1892); Möbius, Notarissia 5: 1071 (1890); Drouet, Amer. Jour. Bot. 24: 603 (1937).—Specimens seen: BRAZIL: with *Lyngbya aestuarii*, *Glaziou 13375* (Ber). CEARÁ: Source: roadside pool near Primavera, S. Wright, 29 Oct. 1937 (D, F, N).

MICROCOLEUS PALUDOSUS (Kütz.) Gom., ibid. 15: 358 (1892).—The one specimen is one of those mentioned by Dickie, Jour. Linn. Soc. Bot. 18: 127 (1880), as *Hypheothrix lutescens* [see Drouet, ibid. 600, footnote 4]: PARÁ: Lagoa Triplex, Rio Trombetas, *Trail 88*, 30 Jan. 1874 (BM).

MICROCOLEUS LACUSTRIS Parl. ex Gom., ibid. 15: 359 (1892).—For other reports of this species in the Brazilian flora, see Drouet, ibid. 603 (1937). The specimens cited here form in part the bases for the reports of *Oscillaria tenuis* var. *calida* and *Hypheothrix lutescens* by Dickie (loc. cit.) [see Drouet, ibid. 600, footnote 4]: AMAZONAS: bottom of small pool on clay rock, Barreiras de Hyutanaham, Rio Purús, *Trail 72*, 23 Sept. 1874 (BM); on moist earth at Sobral, Rio Purús, *Trail 17*, Sept. 1874 (BM); on mud in road near Manáos, *Trail 41*, 12 June 1874 (BM).

SYMPLOCA MURALIS Kütz. ex Gom., ibid. 16: 112 (1892). *S. muralis* var. *minor* Gardn., Univ. California Publ. Bot. 14: 6 (1927).—Here are placed two of the specimens which Dickie (loc. cit.) reported as *Oscillaria tenuis* var. *calida* [see Drouet, loc. cit.]: PARÁ: on a dead log in an ingarapé near Obidos, *Trail 138*, 24 Jan. 1874 (BM). AMAZONAS: on moist earth at foot of a tree, Paricatuba, Rio Purús, *Trail 57*, 8 Sept. 1874 (BM).

SYMPLOCA MUSCORUM (Ag.) Gom., ibid. 16: 110 (1892). *Phormidium Corium* var. *capitatum* Gardn., Univ. California Publ. Bot. 14: 4 (1927).—Other Brazilian reports of this species are mentioned in Drouet, Amer. Jour. Bot. 24: 604 (1937). Among other specimens cited here is the one which Dickie, Jour. Linn. Soc. Bot. 18: 126 (1880), recorded as *Anabaena chilensis*: AMAZONAS: São Paulo, Rio Solimões, *Trail* (BM); on damp soil, mouth of Rio Sasso, *Trail 6*, Feb. 1875 (BM); on mud, bank of Rio

Madeira 25 mi. below Santo Antonio, *Trail 20*, 21 May 1874 (BM).

PLECTONEMA NOSTOCORUM Born. ex Gom., ibid. 16: 102 (1892).—One specimen: CEARÁ: in a fish tank, Fortaleza, S. Wright, 28 Apr. 1937 (D, N).

**LYNGBYA AESTUARIUM* (Mert.) Liebm. ex Gom.—The specimen cited here is the basis of the report of this species from Brazil by Möbius, Notarissia 5: 1071 (1890): BRAZIL: *Glaziou 13375* (Ber).

LYNGBYA LUTEA (Ag.) Gom., ibid. 16: 141 (1892). *Oscillaria sordida* Dickie ex Forti, Syll. Myxophyce. 192 (1907); Dickie (as *Oscillaria*), Jour. Linn. Soc. Bot. 14: 358 (1874).—The type of *O. sordida* contains very few sheathed filaments of a form which is apparently *Lyngbya lutea*: PEDRAS DE SÃO PAULO: from stagnant rock pool, H. Moseley, Challenger Expedition (TYPE of *Oscillaria sordida*, BM).

**LYNGBYA PUTEALIS* Mont. ex Gom.—The specimens listed here are all of somewhat smaller dimensions than those described by Gomont; among them I place the material upon which Dickie, ibid. 18: 127 (1880) [see Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937)], based his report of *Hypheothrix cyanea*: CEARÁ: Russas: in outlet of Açude S. Antonio das Russas, *Drouet 1427*, 17 Sept. 1935 (D). Redenção: under rushing water of small dam, Açude Acaape do Meio, *Drouet 1423*, 14 Sept. 1935 (D, F, N). Fortaleza: creek at Praia Jurema, *Drouet 1339*, 4 Aug. 1935 (BM, D, F, M, N, R, Y); in lagoon, Parque da Independência, *Wright 1540*, 15 Aug. 1935 (D, F, N, R). PARÁ: forming a web on roots over which water was trickling, Tonantins, *Trail 104*, 23 Nov. 1874 (BM).

LYNGBYA DIGUETTI Gom. in Hariot, Jour. de Bot. 9: 160 (1895); Drouet, Rhodora 40: 234, 235, footnotes (1938).—Here are to be placed the specimens reported by Borge as *L. Kuetzingii* in Ark. f. Bot. 19(17): 4 (1925) [see Drouet, Amer. Jour. Bot. 24: 604 (1937)] and by Drouet (loc. cit.) as *L. Lagerheimii* (as to specimens cited from Pará). Material seen: MATTO GROSSO: zwischen Pontederiaceen bei São Luiz de Cáceres, F. C. Hoehne 38, Jan. 1914 (S). CEARÁ: Maranguape: Açude São Bento, P. de Azevedo 1543, 5 Aug. 1935 (BM, D, F, M, N, R). PARÁ: Museu Paraense, Belém, *Drouet 1275* (D, F, L, M, N, R, S, Y).

LYNGBYA EPIPHYTICA Hieron. apud Lemm., Ark. f. Bot. 2(2): 103 (1904).—One specimen: CEARÁ: on filaments of *Tolypothrix distorta* floating in Açude Forquilha, 18 km. east of Sobral, S. Wright, 26 Oct. 1937 (D).

**OSCILLATORIA PRINCEPS* Vauch. ex Gom.—The specimens here are those which Dickie recorded in Jour. Linn. Soc. Bot. 18: 126, 127 (1880), under the names *O. princeps*, *Chthonoblastus oligothrix*, and *Hydrocoleum thermale* [see Drouet, Amer. Jour. Bot. 24: 600, footnote 4 (1937)]: AMAZONAS: clear stream, Igarapé de Manáos, *Trail 17*, 14 Aug. 1874 (BM); pool in forest behind Parauary, Rio Solimões, *Trail 102*, 20 Oct. 1874 (BM); stream at Tabatinga, *Trail 102*, 30 Nov. 1874 (BM).

**OSCILLATORIA LIMOSA* Ag. ex Gom.—The specimen cited here was interpreted by Dickie (loc. cit.) as *Phormidium parallelum* [see Drouet, loc. cit.]: PARÁ: Ponta Jaguarari, Rio Tapajóz, *Trail*, 12 Jan. 1874 (BM).

**OSCILLATORIA TENUUS* Ag. ex Gom.—The following specimens are recorded by Dickie (loc. cit.) [see Drouet, loc. cit.]: as *O. tenuis* var. *calida* and *Phormidium australe*: PARÁ: muddy puddles in road near Obidos, *Trail*, 10 Feb. 1874 (BM). AMAZONAS: Retenção, Rio Purús, *Trail*, 3 Oct. 1874 (BM).

**OSCILLATORIA SPLENDIDA* Grev. ex Gom.—Additional material, from AMAZONAS: on rotten wood in stream, Maracapurá, *Trail* 89, 6 Sept. 1874 (BM).

**OSCILLATORIA FORMOSA* Bory ex Gom.—Additional material, that reported by Borge under this name in Ark. f. Bot. 19(17): 4 (1925), rather poorly preserved: MATTO GROSSO: Regenwasser-See bei São Luiz de Cáceres, F. C. Hoehne 74, Jan. 1914 (S).

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RELATION OF SULPHATE TO SELENIUM ABSORPTION BY PLANTS¹

Annie M. Hurd-Karrer

TOXICITY STUDIES with both soil and water cultures have demonstrated that injury to wheat from sodium selenate is a function of the available sulphate concentration (Hurd-Karrer, 1934). The few plant analyses that have been made have indicated that the higher the concentration of sulphur available to the plant the lower the absorption of selenium (Hurd-Karrer, 1935, 1937c; Hurd-Karrer and Kennedy, 1936). This relationship is demonstrated in the present paper by more extensive analyses of material grown in both nutrient solutions and in soils.

The method of selenium analysis was that reported by Robinson, Dudley, Williams, and Byers (1934) with some modifications, especially in the case of analyses made by Dr. L. Greathouse, who used a new method of separation, digesting the plant material with bromic acid. The method for sulphur was that recommended by the Association of Official Agricultural Chemists for plant material. Results are expressed as parts per million of the air-dry weights of the plants.

WATER CULTURES.—Seed of Hard Federation wheat was germinated in quartz sand kept moist with distilled water, and the young seedlings transferred after 6 days, 3 to each of the 600 cc. wide-mouthed Erlenmeyer flasks containing the nutrient solutions. Graded concentrations of sodium selenate were set up in nutrient solutions of different sulphate concentrations, like those described earlier (Hurd-Karrer, 1934). The series of plants at each sulphate level was terminated at a selenium-sulphur ratio of 1:4, where it was known from previous experiments that the plants would sustain severe but not fatal selenium injury.

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After 8 weeks the plants were cut, weighed, and air-dried for selenium and sulphur analyses. Averages of duplicate determinations² and the records of leaf chlorosis are given in table 1. The degree of chlorosis indicated by a single plus sign was associated with slight stunting of the plants, while the severe chlorosis at the 1:4 ratio, indicated by a triple plus sign, was associated with about a 50 per cent reduction in size in each case.

The selenium in the plants increased with that in the nutrient solution at each of the four sulphate levels. At each selenium level it decreased with increasing sulphate in the solution. The decreasing magnitude of this effect as the concentration of sulphate increased suggests an approach to a limit beyond which the selenium intake cannot be reduced by sulphate. Such a limit is also suggested by the data of other experiments (cf. fig. 2). However, interpretation is complicated by the fact that the 192 p.p.m.-sulphur solution was in itself toxic, as shown by the control plants without selenium, which were about three-fourths the normal size; and stunting might be expected to produce disproportionately high concentrations of selenium.

The sulphur determinations, made on aliquots of the same dried samples analyzed for selenium, also revealed some interesting trends. The plants often took up the least sulphur from the solution with the next to the lowest sulphur concentration, 32 p.p.m. Above this point, the absorbed sulphur increased regularly with that in the solution at the lower selenium levels but not at the higher ones. At each of the three lower sulphate levels sulphur absorption increased regularly with increasing selenium in the nutrient solution. An explanation is not apparent, the effect

² The two selenium duplicates were determined by different methods, turbidimetrically and by titration. The trends were the same by both methods, but the former gave consistently lower values. Since the titration method seemed likely to be the more accurate for the range of concentrations involved, it is probable that the averages are somewhat too low, excepting the high value in each sulphur series, which was determined gravimetrically.

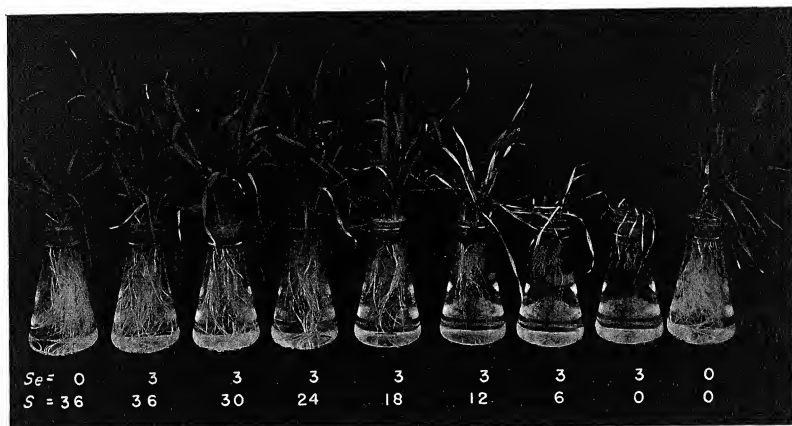


Fig. 1. Demonstration of sulphur-selenium antagonism with wheat grown 3 weeks in nutrient solutions containing 3 p.p.m. selenium (as sodium selenate) and 0 to 36 p.p.m. sulphur (as magnesium sulphate), as indicated by figures under flasks.

being too large to be due to any possible interference of the selenium in the tissues with the sulphur determination.

The amount of selenium in the plants and the degree of visible injury were obviously determined by the selenium/sulphur ratio in the nutrient solution. Excepting the results with the highest sulphate solution, an external ratio of one part of selenium to four of sulphur produced plants that were all similarly chlorotic and contained approximately equal amounts of selenium, about 1300 p.p.m., regardless of the absolute amounts of selenium available; an external ratio of one to eight produced only slight chlorosis, with 322, 429, and 538 p.p.m. selenium, respectively, in the tissues of the three lots of plants available for com-

parison; an external ratio of one to sixteen produced plants that were all normal in appearance but contained 146, 165, and 188 p.p.m. selenium, respectively.

The upward trend in each of these groups of figures shows that the selenium absorbed at a given external ratio increases somewhat with the absolute amount available, which would indicate that the actual selenium concentration is a factor in absorption at equal selenium/sulphur ratios. Such an effect of absolute selenium concentration at equal ratios would account for the observation in some of the experiments that there is somewhat greater stunting at a given external ratio with the larger amounts of selenium.

TABLE 1. *Selenium and sulphur absorption by wheat in nutrient solutions containing the two elements in various amounts and proportions.^a*

Selenate selenium in nutrient solutions	Degree of leaf chlorosis with the following p.p.m. sulphate sulphur available—				Selenium in plants with the following p.p.m. sulphate sulphur available—				Sulphur in plants with the following p.p.m. sulphate sulphur available—			
	16	32	96	192	16	32	96	192	16	32	96	192
<i>p.p.m.</i>					<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>	<i>pct.</i>
0	0	0	0	0	0	0	0	0	.540	.430	.720	1.105
1	0	0	0	0	146	76	38	12	.775	.710	.830	1.125
2	+	0	0	0	322	165	50	48	.880	.840	.950	1.095
4	+++	+	0	0	1333	429	113	103	1.195	.975	1.090	1.100
6		++	0	0		746	188	143		1.100	1.075	1.235
8		+++	0	0		1280	328	278		1.180	1.010	1.145
12			+	0			538	396			1.170	1.155
24			+++	+			1253	1004			1.225	1.335
48				+++				2046				1.470

^a Selenium in plants determined by Dr. Lucien Greathouse, sulphur by Mr. J. B. Martin.

To facilitate comparison of the sulphur/selenium ratio in the plant with that in the solution, the ratios, calculated from the data of table 1, are summarized in table 2. Again excepting some discrepancies with the highest sulphur solution, the ratio of sulphur to selenium in the plants was similar for each external

hence those of each sulphur level were grouped into two composite samples, one comprising the plants from solutions containing ten or less times as much sulphur as selenium, all of which were chlorotic, and the other comprising plants from solutions having twelve or more times as much, all of which appeared normal. The selenium concentrations of the nutrient solutions producing each sample were averaged to represent roughly the selenium available in each case.

The data of table 3 show that the plants' selenium content was roughly proportional to that of the nutrient solution at each sulphur level; and their sulphur content increased with increasing amounts of sulphate in the solution, as would be expected. More difficult to explain is the fact that, as in the preceding experiment, the amount of sulphur absorbed at each of the five sulphate levels was markedly higher in the solution containing the higher concentration of selenium.

The effect of the sulphate in the nutrient solution on the absorption of selenium is not at once apparent in these data because the concentrations of selenate were not the same at the different sulphur levels. The effect can be demonstrated, however, by calculating the amount of selenium absorbed by the plants at each sulphur level per unit p.p.m. of selenium in the solution. Averages of the two such calculations possible at each of the five sulphur levels, 0, 10, 32, 96, and 192 p.p.m., were 450, 311, 96, 57, and 45 p.p.m. selenium, respectively. Thus as the available sulphur increased, the selenium intake per unit of selenium in the solution decreased.

The chlorotic plants in table 3 were all produced by sulphur/selenium ratios of between 3 and 5 in the nutrient solutions. They had an average sulphur/selenium ratio of 9.4 in their tissues, which agreed closely with the corresponding ratio obtained for such plants in the preceding experiment (table 2). The two experiments indicated that, regardless of the absolute amount of selenium available, a fourfold proportion of sulphur to selenium in the nutrient solution resulted in an approximately ninefold proportion in

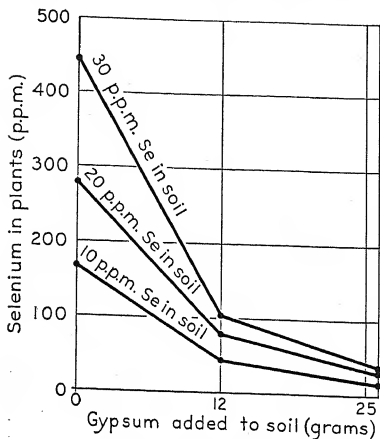


Fig. 2. Effect of gypsum on the selenium content of mature wheat plants in soil treated with 10, 20, and 30 p.p.m. selenium as sodium selenate.

sulphur-selenium ratio, regardless of the absolute amounts of the elements available. It was nine or ten where the ratio in the solution was four, somewhat over twenty where the ratio in the solution was eight, and somewhat over fifty where the ratio in the solution was sixteen. The fact that the internal and

TABLE 2. The proportionate amounts of selenium and sulphur in the plant in relation to those in the nutrient solution, as calculated from the data of table 1.

S/Se ratio in the nutrient solution	S/Se ratio in the plants with nutrient solutions containing—			
	16 p.p.m. S.	32 p.p.m. S.	96 p.p.m. S.	192 p.p.m. S.
4	9.0	9.2	9.8	7.2
8	27.3	22.7	21.7	12.8
16	53.1	50.9	57.2	29.2
32		93.4	—	86.4
48			190.0	106.8
96			218.4	228.1

external ratios are not equal indicates unequal absorption rates of the two elements. The rate of absorption of sulphur was greater than that of selenium.

A few additional analyses are available from another experiment (table 3). There was insufficient material for analyses of plants of individual cultures,

the plants; and this proportion resulted in the degree of chlorosis that consistently characterized plants grown in such solutions.³

³ It is recognized that the ratios probably obtain only within the moderate concentration limits of the experiments.

TABLE 3. *Selenium and sulphur absorption by wheat in relation to growth in nutrient solutions containing the two elements in various amounts and proportions.^a*

Nutrient solution		Condition of leaves	Composition of plants		Ratio of sulphur to selenium in—	
Sulphate sulphur	Selenate selenium (average)		Selenium	Sulphur	Nutrient solution	Plants
p.p.m.	p.p.m.		p.p.m.	per cent.		
0 ^b	.03	Normal	12	215	—	179.2
0 ^b	.5	Chlorotic	250	243	—	9.7
10	.5	Normal	175	334	20.0	19.1
10	3.5	Chlorotic	950	586	2.9	6.2
32	1.5	Normal	125	417	21.3	33.4
22	7.7	Chlorotic	830	374	4.2	10.5
96	8.5	Normal	550	815	11.3	14.8
96	20.7	Chlorotic	1030	1,066	4.6	10.7
192 ^c	14.0	Normal	700	1,086	13.7	15.5
192 ^c	39.3	Chlorotic	1550	1,527	4.9	9.9

^a Selenium in plants determined by Mr. A. Van Kleeck, sulphur by Mr. J. B. Martin.^b No sulphate was added to this solution, but it was found to contain 0.9 p.p.m. sulphur on analysis.^c All the plants of this series were slightly stunted.

Leaf chlorosis, the criterion of selenium injury that has been most used in these and preceding experiments, is associated with a proportionate degree of stunting of both roots and tops. The plants of an experiment summarized in table 4 and illustrated in figure 1 show this correlation. The plants were grown five weeks in nutrient solutions⁴ containing 3 p.p.m.

TABLE 4. *The toxicity of 3 p.p.m. selenium, as sodium selenate, in nutrient solutions containing different concentrations of sulphate sulphur.*

Composition of nutrient solutions			Chlorosis	Dry weight of tops (3 plants)
Sulphate sulphur	Selenate selenium	Se/S		
p.p.m.	p.p.m.			grams
36	0	—	0	2.40
36	3	1:12	0	2.55
30	3	1:10	+	2.32
24	3	1:8	++	2.10
18	3	1:6	+++	1.50
12	3	1:4	++++	1.02
6	3	1:2	+++++	0.19
0	3	—	Plants dead	0.07
0	0	—	0	1.75

⁴The solutions of this experiment were somewhat different from those used previously. Two stock solutions containing 36 p.p.m. sulphur (A) and no sulphur (B) were mixed in different proportions to obtain intermediate concentrations. Both solutions contained, in 19 liters, 107 cc. of M/5 KH₂PO₄ and of M/2 Ca(NO₃)₂, plus 100 cc. M/60 iron citrate (more of which was added weekly to the cultures). Solution A also contained 107 cc. M/5 MgSO₄. Solution B the same amount of M/5 Mg(NO₃)₂. They thus differed with respect to nitrate, but control plants showed no appreciable effect of this difference on the growth of the seedlings. The acidity of the solutions, about pH 5.5, was too high for best growth, so they were brought almost to neutrality with sodium hydroxide.

of selenium and different quantities of sulphate. Injury from the selenium as shown by both leaf color and plant weights was completely prevented by a twelvefold concentration of sulphur, as in previous experiments (Hurd-Karrer, 1934). But such apparently uninjured plants have always been found to contain selenium. It can be predicted from the results of tables 1 and 3 that the uninjured plants of table 4 contained at least 200 p.p.m.

The question arises as to how much selenium the tissues must contain before the plant shows visible injury. In table 1, plants grown in the low-sulphur solution showed the snow-white selenium chlorosis with a selenium content of 322 p.p.m., while those in the high-sulphur solution were normal in appearance with 396 p.p.m. selenium. In table 3, plants grown in the solution made up without sulphate were chlorotic with a selenium content of 250 p.p.m., while those in the high-sulphur solution took up 700 p.p.m. selenium without visible injury. Some internal factor, probably the relative amount of sulphur in the tissues, as suggested previously (Hurd-Karrer, 1935), determined the toxicity of the absorbed selenium. Consequently, prediction of the selenium content of the plant from the degree of injury is impossible unless the plants are grown under conditions of equal sulphur availability.

SOIL CULTURES.—Hard Federation wheat was grown in the greenhouse in sterilized Keyport clay loam in 10-inch pots containing 6 kg. soil on a layer of gravel. The total natural sulphur content of this soil was .06 per cent, the water soluble sulphur .005 per cent. Solutions containing the desired quantities of selenate and of the soluble sulphates were thoroughly mixed into the soil of each pot. Elemental sulphur and gypsum were added dry, of course. Thirteen plants were grown to maturity in each pot.

Notes on comparative degrees of chlorosis, indicated by plus signs in the tables, were made when the

TABLE 5. *Effect of gypsum on absorption and toxicity to wheat of selenium added to Keyport clay loam as sodium selenate.^a*

Selenium added to soil	CaSO ₄ .2H ₂ O added per 6 kg. soil	Chlorosis on young plants	Condition of plants				Selenium in plants (duplicate analyses)		
			Yield per pot of 13 mature plants				A	B	
			No. of heads		Weight of				
			Fertile	Sterile	Straw	Grain			
<i>p.p.m.</i>	<i>grams</i>					<i>grams</i>	<i>grams</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
10	0	0	20	2	35.5	15.9		165	170
10	12	0	36	1	70.6	34.4		50	40
10	25	0	21	0	45.1	27.5		18	17
20	0	++	24	1	31.9	14.1		280	280
20	12	0	28	0	53.0	26.0		80	80
20	25	0	26	0	43.3	26.7		35	30
30	0	+++	3	17	15.6	1.4		545	—
30	12	0	18	1	38.3	22.7		105	—
30	25	0	34	0	43.8	24.2		40	40

^a Selenium in plants determined by Dr. Lucien Greathouse.

plants were young. At maturity, the number of fertile and sterile heads and the dry weights of straw and of grain were recorded. Comparisons are made with reference to control plants grown in the same soil without selenium.

Table 5 shows the effect of an excess of sulphate in the form of gypsum on the growth and selenium intake of the plants. The increased yields of grain and straw produced by the applications of gypsum at each selenium level were obviously correlated with decreased intake of selenium. The regularity with which the selenium in the plants decreased with increasing sulphate in the soil is illustrated in figure 2.

A similar experiment with ammonium sulphate is summarized in table 6. At each selenium level the addition of ammonium sulphate reduced the selenium entering the plants. Associated with reduction in selenium absorption were corresponding reductions in plant injury—decreased chlorosis and increased fertility. With the lowest soil selenium, 15 p.p.m., the high sulphate application completely inhibited all symptoms of injury, but with 30 p.p.m. selenium it

merely improved the condition of the plants, the considerable amount of selenium they still contained causing rather severe injury.

When 20 per cent of ordinary sand was added to the clay loam soil, 10 p.p.m. selenium instead of being non-toxic produced extreme chlorosis, which was completely prevented by the application of 6 grams of ammonium sulphate. The analyses of these plants, the first group in table 7, do not show the progressive decrease in selenium intake with increasing sulphate that was expected from the progressive improvement in their appearance. However, such a decrease is evident in the figures for the other two groups in table 7—i.e., with 20 and 30 p.p.m. selenium, respectively.

A series with elemental sulphur instead of sulphate, added to Keyport clay loam containing 20 p.p.m. selenium, gave the following results. Without the added sulphur, injury was severe, and the mature plants contained 240 p.p.m. of selenium. With one gram of sulphur in 6 kg. of soil, symptoms of injury were reduced, but the single analysis available showed

TABLE 6. *Growth and selenium content of wheat plants in selenized Keyport clay loam with applications of ammonium sulphate.^a*

Selenate selenium added (p.p.m. Se)	Grams of (NH ₄) ₂ SO ₄ per 6 kg. soil	Chlorosis on young plants	Condition of plants				Selenium (p.p.m.) in plants (duplicate analyses)	
			Yield per pot of 13 mature plants					
			No. of heads		Weight (grams) of—		A	B
			Fertile	Sterile	Straw	Grain		
15	1	+	46	26	94.8	28.7	168	175
15	4	0	45	6	97.2	39.3	33	37
20	1	++	26	56	77.8	6.7	250	210
20	4	0	33	5	75.5	26.5	50	50
30	1	+++	20	57	74.0	6.0	400	415
30	4	++	36	25	67.2	21.3	290	300

^a Selenium in plants determined by Dr. Lucien Greathouse.

TABLE 7. *Growth and selenium content of wheat plants in selenized sandy loam with and without applications of ammonium sulphate.^a*

Selenate selenium added (p.p.m. Se)	Grams of (NH ₄) ₂ SO ₄ per 6 kg. soil	Chlorosis on young plants	Condition of plants				Selenium in plants (p.p.m.)
			Yield per pot of 13 mature plants				
			No. of heads		Weight (grams) of—		
			Fertile	Sterile	Straw	Grain	
10	0	+++	26	5	39.8	17.7	197
10	2	++	33	9	63.0	19.0	227
10	4	+	51	8	79.5	30.0	154
10	6	0	45	7	72.3	37.7	198
20	0	+++++	4	25	13.8	1.2	360
20	2	+++	19	41	57.3	6.7	320
20	4	++++	30	18	51.4	19.6	295
20	6	++	40	11	56.7	25.3	250
30	0		Plants dead in seedling stage				
30	2	+++++	5	39	28.3	0.7	790
30	4	+++	15	31	42.8	7.2	450
30	6	+++	38	6	48.2	20.8	370

^a Selenium in plants determined by Dr. Lucien Greathouse.

no reduction in selenium intake. With 3, 5, and 7 grams of sulphur, the selenium content was reduced to 170, 140, and 120 p.p.m., respectively. Visible injury was completely inhibited by the 5- and 7-gram applications.

The critical selenium/sulphur ratios for injury to plants grown in nutrient solutions cannot be expected to hold for plants grown in soil unless the soil analyses indicate the relative availability of these elements in the soil solution in immediate contact with the roots. The ordinary soil analysis is not of this solution and may give little information as to the concentrations actually available to the plant. Soils bind selenium (Beath, 1937; Beath, Eppson, and Gilbert, 1935, 1937); Franke and Painter, 1937) and this binding capacity varies with different soils. With non-uniform vertical distribution of selenium (Byers, 1935, 1936; Byers and Knight, 1935; Beath, Eppson and Gilbert, 1937), determination of the amounts available to the plants is especially difficult. Therefore reference to the critical ratios as though they had been obtained with soils (Franke and Painter, 1937; Strock, 1935) is misleading. Moreover, it should be noted that they apply to selenate selenium only. All forms of selenium are not equally available to plants, nor is their absorption similarly affected by sulphur (Martin, 1936; Beath, 1937; Beath, Eppson and Gilbert, 1937; Hurd-Karrer, 1937c).

To determine whether other salts besides sulphates affect selenium absorption, wheat plants were grown with sodium selenate (20 p.p.m. selenium) in soil treated with the sulphate, phosphate and nitrate of ammonium, calcium, potassium, and magnesium in equivalent amounts. All the plants became chlorotic and stunted, with yields of grain reduced by at least 50 per cent, excepting those grown with the four sulphates, which were indistinguishable from the control plants without selenium (fig. 3). It was evident

that neither the nitrate and phosphate ions, nor the cations—ammonium, calcium, potassium and magnesium—had any appreciable effect on selenium toxicity.

The question next arose as to whether the effect of sulphur on selenium absorption obtains with plants other than wheat. Accordingly, several different crops were grown in Keyport clay loam treated with calcium selenate at a rate of 3 p.p.m. selenium, a non-toxic concentration in this soil. One end of a greenhouse bench, separated from the rest by a buffer plot of dry soil, received in addition to the selenate an

TABLE 8. *Absorption of selenium by crop plants from 3 p.p.m. in the form of calcium selenate added to Keyport clay loam with and without applications of sulphur.^a*

Crop	Selenium (p.p.m.) in plants from soil—			
	Without sulphur treatment		With sulphur treatment	
	A	B	A	B
Young Plants				
Cabbage	310	370	20	20
Turnip	260	220	20	20
Spinach	40	60	8	—
Old plants				
Broccoli leaves.	230	210	70	80
Cabbage leaves.	190	170	70	60
Pea leaves	60	60	50	40
Pea seed	60	40	20	30
Corn leaves ...	60	60	30	20
Onions	36	—	10	—
Lettuce	70	30	70	—

^a Selenium in plants determined by Mr. R. B. Deemer and Mr. R. F. Gardiner.



Fig. 3. Wheat plants grown with 20 p.p.m. selenium added as sodium selenate to Keyport clay loam treated with the following salts: First row, left to right, NH_4NO_3 , KNO_3 , $\text{Ca}(\text{NO}_3)_2$, $\text{Mg}(\text{NO}_3)_2$. Second row, $(\text{NH}_4)_2\text{HPO}_4$, $\text{K}_2\text{H}_2\text{P}_2\text{O}_7$, $\text{CaH}_2(\text{PO}_4)_2$, control with selenate only. Third row, $(\text{NH}_4)_2\text{SO}_4$, K_2SO_4 , CaSO_4 , MgSO_4 . Chlorosis and stunting severe on all plants except those of the third row treated with sulphates.

application of elemental sulphur at the rate of one part in 1200 of air-dry soil. Comparison of the weights of the plants at the time of cutting with those of control plants grown without selenium in another bench showed that growth was in no case reduced by the selenium.

Table 8 shows that the selenium absorption of all the crops except lettuce (for which the data are inadequate) was reduced by excess sulphur. The effect was greater in the case of young plants than in that of old ones, as found previously with wheat (Hurd-Karrer and Kennedy, 1936).

DISCUSSION.—To account for the manifestations of the selenate-sulphate antagonism one may assume that the plant's synthesis of sulphur compounds establishes a metabolic gradient to which selenium as well as sulphur responds by virtue of chemical similarity; and that harmful substitution^a of selenium

in compounds normally formed with sulphur occurs in proportion to the relative availability of the two elements. The effect of sulphur on the toxicity of selenium then becomes simply a mass effect, a type of antagonism which might appropriately be termed "mass antagonism."

The idea that selenium can substitute for sulphur in plant metabolism was first proposed by Cameron (1880), who thought that selenium injury to plants might be the result of utilization of selenium instead of sulphur in synthesized compounds. Subsequently, several investigators tried substituting selenium for sulphur in nutrient solutions (Awschalom, 1921; Brenner, 1916; Levine, 1925) but discovered only the extreme toxicity of selenium. That this toxicity now

phyll body is correct, it might be expected that magnesium salts of the stronger acids would exert a noxious action. The lime as the stronger base would in such a case combine with the acid of the magnesium salt, while magnesia would enter into the place which the lime had occupied in the organized structures, the capacity for inhibition would thereby be altered, and a disturbance of the structure would result which would prove fatal. On the other hand, judging from the laws of the action of masses, it would naturally be inferred that an excess of lime salts would remedy the evil effects by making the reverse process possible."

^a Substitution in the sense of alternative utilization in syntheses of organic compounds, not in the sense of replacement in molecules already formed, is meant. The following conception of the calcium-magnesium antagonism as a manifestation of relative availability of substitutable ions is given by Loew (1903, p. 49): "If the writer's view that a calcium-protein compound participates in the organized parts of the nucleus and chloro-

proves to be a function of sulphur availability seems consistent with Cameron's idea of harmful substitution.

Besides various quantitative aspects of the antagonism, that seem difficult to explain on any other basis, additional observations in accord with the assumption are: First, the nature of the snow-white chlorosis on the new leaves of such plants as wheat, barley, and rye,⁶ and its non-occurrence on the older leaves already formed at the time the selenium is made available, suggest non-formation of chlorophyll rather than destruction of that already formed. Displacement of the plastids in the meristematic tissue might be attributed to substitution of selenium for sulphur in the synthesis of their protein molecules. Second, occurrence of the selenium of wheat grain in intimate association with the proteins (Nelson et al., 1933; Franke, 1934; Horn et al., 1936) and the sulphur-containing amino acids (Painter and Franke, 1936; Jones et al., 1937) might be the result of substitution in these compounds, although the analyses of Jones et al. fail to show an exact correlation between the selenium and cystine contents. Third, comparative analyses of different kinds of plants have shown that selenium absorption is directly correlated with the sulphur requirement (Hurd-Karrer, 1937b). Selenium intake apparently depends on the sulphur gradient established by the plant's tendency to form sulphur-containing compounds. Fourth, the recently determined facts that phosphate inhibits arsenic toxicity, potassium inhibits rubidium toxicity, and calcium inhibits strontium toxicity, all with reproducible critical ratios, were predicted from the idea that the effect of sulphur on selenium absorption is a mass effect by virtue of chemical relationship (Hurd-Karrer, 1936b; 1937a).

Lack of identity between the selenium-sulphur ratio in the nutrient solution and in the plant (table 2) indicates that the rates of absorption of selenium and sulphur are not equal, proportionately more sulphur than selenium being taken into the plant. Moreover, the literature on permeability shows that equal penetration of chemically related ions cannot be assumed. Where inequality can be attributed to physical properties of the root membranes, there might still remain the possibility of sufficient chemical and physiological unselectivity as to account for the type of antagonism here discussed.

This explanation is based on circumstantial evidence, but it has at least provided a unifying conception for some of the various observations and has

⁶ In numerous experiments with both soil and nutrient solutions, the snow-white chlorosis which was always much in evidence on these three crops, and occasionally on sorgo, was entirely lacking on all the other crops grown. On such plants as buckwheat, and in fact on all the broad-leaved plants, the discoloration was yellowish like that ordinarily produced by selenite, never snow-white. It was not determined whether this yellowish chlorosis spread over green leaf areas after leaf emergence. It may also be of interest to note here that the writer has never been able to produce the snow-white chlorosis with selenites, even on wheat, at ordinary sulphur levels (Hurd-Karrer, 1937c).

led to predictions subsequently verified by experiment. An explanation postulating reduction of the selenium in the substratum by hydrogen sulphide evolved from sulphur compounds (Moxon and Franke, 1935) would seem to leave the reproducible quantitative aspects of the antagonism unexplained. Internal precipitation or inactivation of the selenium through formation of a seleno-sulphur compound fails to account for the fact shown by the plant analyses that the total selenium absorbed decreases with increasing available sulphate instead of increasing.

That the reduction in selenium absorption in the presence of excess sulphate has any wide practical value for agriculture in the naturally seleniferous soil areas seems doubtful. The chief sources of selenium in the naturally toxic soils do not seem to be selenates but selenites (Williams and Byers, 1936) and organic selenium from plant residues (Beath, 1937; Beath, Eppson, and Gilbert, 1937). Absorption of organic selenium is reported to be unaffected by sulphur (Beath, 1937; Beath, Eppson, and Gilbert, 1937); and absorption of selenite is less affected than is that of the selenate used in the present experiments (Hurd-Karrer, 1937c). However, a rather large reduction in selenium absorption by wheat as a result of applying sulphur and ammonium sulphate to soil treated with potassium selenite has recently been reported (Beath, 1937); and absorption of selenite selenium by *Astragalus* has been shown to decrease as the sulphate sulphur in the nutrient solution increases, within limits (Trelease and Trelease, 1938).

Byers (1935) and Byers and Knight (1935) have reported some instances of field vegetation with disproportionately low selenium content in regions of abundant sulphate supply. But Franke and Painter (1937)⁷ have concluded that sulphur and gypsum treatments of naturally seleniferous soils under field conditions in South Dakota are ineffective, although conditions for oxidation of the sulphur in their experiments were evidently unfavorable. Certainly on any soil containing selenate selenium (Byers, 1936, p. 72; Williams and Byers, 1936) and not saturated with sulphates it seems reasonable to expect from the evidence that the toxicity of the vegetation to animals can be reduced by the use of sulphur. Whether or not this reduction will render the vegetation non-toxic or non-lethal obviously depends on the quantity and kind of selenium compounds in the soil. With but 2 p.p.m. added as sodium selenate to outdoor soil plots, all evidence of toxicity of wheat grain to rats was prevented by both sulphate and sulphur applications (Hurd-Karrer and Kennedy, 1936).

Beath and his associates have reported that some kinds of crop plants, including barley, do not take up

⁷ A statement in this paper that reduction in the selenium content of grain brought about by sulphur was less in the writer's more recent experiments (Hurd-Karrer and Kennedy, 1936) than in those reported earlier (Hurd-Karrer, 1935) can be clarified by emphasizing that the earlier analyses were not of grain but of green plants. Furthermore, less selenium in the plants of the later experiments is accounted for by the fact that less selenium was added to the soil (Keyport clay loam).

the inorganic selenium of the naturally seleniferous shales of Wyoming (Beath, Draize, Eppson, Gilbert, and McCreary, 1934), at least to the extent of becoming toxic to animals (Beath, Draize and Gilbert, 1934; Beath, Eppson, and Gilbert, 1935, 1937) but readily absorb the organic selenium returned to the soil by other so-called converter or indicator plants. These observations suggest that there is no appreciable amount of soluble selenate in the Wyoming soils, for the writer has found no plant that fails to take up appreciable quantities of selenate selenium. Barley absorbed it readily, as did alfalfa, whose capacity to absorb much selenium under field conditions has been questioned (Beath, Eppson, and Gilbert, 1935). The extent of absorption by these and all the other crops grown followed the relative sulphur requirement of the species (Hurd-Karrer, 1936a, 1937b).

SUMMARY

Plant analyses have shown that absorption of the selenium of sodium selenate varies directly with the amount available to the plant and inversely with the concentration of sulphate, within limits. Although in both nutrient solutions and soils the selenium absorption was sometimes reduced by excess sulphate to one-tenth that in corresponding low-sulphate cultures, it was never entirely prevented.

The amount of selenium that wheat plants can contain without visible injury depends on their sulphur content. When this was high, 700 p.p.m. of selenium in the tissues produced no visible effect; when it was low the plants were chlorotic with about one-third this amount (table 3).

The analyses show proportionately greater absorption of sulphur than of selenium; e.g., a selenium/sulphur ratio of 1:4 in the nutrient solution produced a ratio near 1:9 in the plants. Such plants were similarly chlorotic and contained about 1300 p.p.m. selenium with absolute amounts available ranging from 4 to 24 p.p.m. (table 1).

The effect of sulphate in reducing the selenium content of the several crop plants analyzed was more pronounced with young green plants than with old ones.

The antagonism is discussed in relation to the idea, first proposed by Cameron (1880), that selenium can be utilized instead of sulphur in syntheses of organic compounds. This idea and the quantitative relations shown by the data have suggested a possible explanation of the effect of sulphur as a mass effect of an element sufficiently similar to selenium as to preclude selectivity.

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A NEW ARIZONA SPECIES OF ECHINOCEREUS¹

Robert H. Peebles

Echinocereus rectispinus sp. nov.—Planta e basi ramosa interdum simplex; caules 15-25 cm. longi 5.0-7.5 cm. diametro conici apice obtusissimi; costae 9-10 humiles undulatae; areolae orbiculatae; aculei fuscii mox cinerei recti et validi aculeus centralis principalis 10-26 mm. longus teres porrectus solitarius vel saepe 1-2 aculeis brevibus centralibus prope apicem areolae, aculei radiales 7-9 adpressi paulo compressi 10-15 mm. longi; petala purpurea ca. 5 cm. longa; baccae globosae; semina lineata-tuberculata.

Type: Peebles No. SF 905 (fig. 1), hills near Nogales, Arizona, elev. 3,900 ft., May 5, 1935, represented by a living plant under cultivation at the U. S. Field Station, Sacaton, Ariz., and by material deposited in the U. S. National Herbarium as No. 1729266.

Other collections: No. SF² 172, Nogales, in 1927; No. SF 225, Patagonia Mts., in 1928; No. SF 890, elev. 3,950 ft., and No. SF 897, elev. 2,700 ft., both Tucson to Redington, in 1935; SF 911 and SF 912, both elev. 3,900 ft., near Nogales, in 1935; all collected in Arizona by the writer.

E. rectispinus is common on the grasslands and in the foothills, at 3,000 to 5,000 feet elevation, from the Santa Catalina Mts., southward and eastward. The species almost certainly ranges into northern Sonora, although we have seen no specimen from Mexico. Britton and Rose³ published a good photograph (their fig. 45) of a flowering plant of this species as a form of *E. Fendleri* (Engelm.) Rümpler. However, *E. rectispinus* is adequately distinguished from *E. Fendleri* by the shorter, stouter, straight, and porrect central spine, by occasional development of

accessory central spines, and by the larger size of the plant. There is no evidence that the two species are connected by intergrading forms, and their geographical ranges appear to be entirely distinct. Typical *E. Fendleri*, to the knowledge of the writer, is confined to northern New Mexico and northern Arizona, where it is rather common at approximate elevations of 5,000 to 7,500 feet. Plants of *E. Fendleri* from this cooler northern region have retained their small size and other essential characteristics when grown for as long as three years under irrigation at Sacaton, in southern Arizona, at an elevation of 1,300 feet.

Echinocereus rectispinus var. *robustus* var. nov.—Caules 2.5-4.5 dm. longi plures graciliores; aculei longiori minus validi, aculeus centralis 2.5-6.0 cm. longus.

Type: Peebles No. SF 896, Tucson to Sabino Canyon, Pima County, Arizona, August 27, 1935, represented by a living plant grown under cultivation at Sacaton, Ariz., and by material deposited in the U. S. National Herbarium as No. 1729267.

Other collections: King & Peebles No. SF 371, hills east of Vail, in 1931; Peebles No. SF 899, Vail, in 1935; Peebles No. SF 900, hills east of Vail, in 1935.

The photograph reproduced in Britton and Rose,⁴ figure 44, represents a characteristic specimen of this variety, which is common in the vicinity of Tucson. As far as known, the accessory central spines always are well developed in var. *robustus*. Although additional material may indicate that var. *robustus* is specifically distinct from *E. rectispinus*, in our series of collections the two appear to intergrade.

On account of the important rôle given to the central spines in taxonomic treatment of the genus *Echinocereus*, spine-clusters of all the species under discussion, with the exception of *E. rigidissimus* (Engelm.) Rose, were arranged in approximately nat-

¹ Received for publication July 27, 1938.

² The letters S.F. indicate living plants in the cactus garden at the U. S. Field Station, Sacaton, Arizona.

³ Britton, N. L., and J. N. Rose. 1922. The Cactaceae. Carnegie Inst. No. 248, vol. 3, p. 37, fig. 45.

⁴ *Ibid.*, p. 36. See footnote 3.

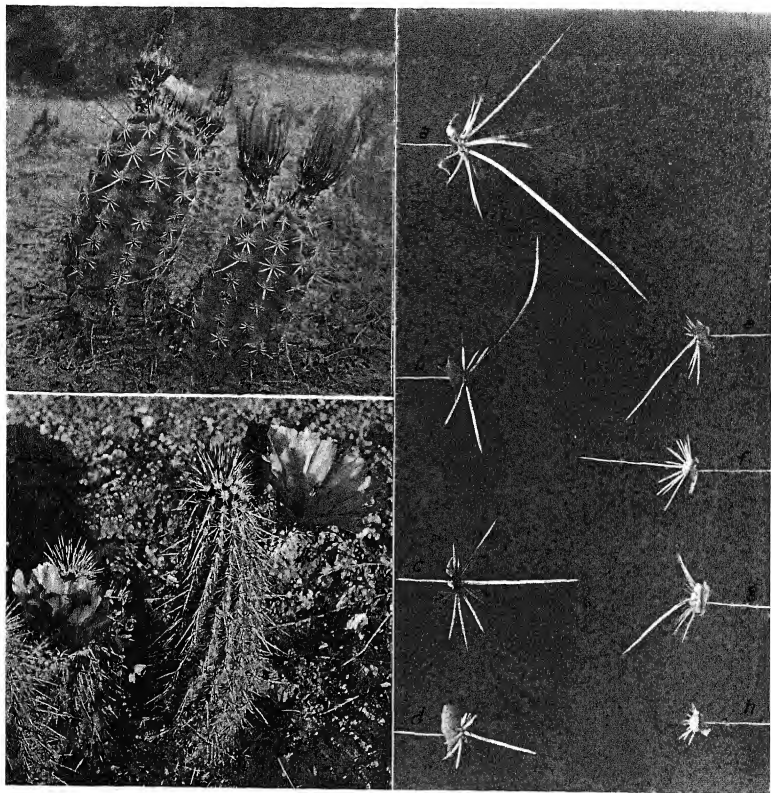


Fig. 1 (upper left). *Echinocereus rectispinus*, Peebles SF 905 (type). Plant 22 cm. high.—Fig. 2 (lower left). This specimen of *E. Boyce-Thompsoni*, Peebles SF 985, collected near Coolidge Dam, is representative of the species as it grows throughout most of its range. Plant 20 cm. high.—Fig. 3 (right). Characteristic arrangement of central spines in (a) *E. Engelmannii*, (b) *E. Fendleri*, (c) *E. rectispinus* var. *robustus*, (d) *E. rectispinus* (typical form), (e, f) *E. Boyce-Thompsoni*, (g) *E. Ledingii*, and (h) *E. Bonkeræ*. (Longest spine shown in fig. 3 is 60 mm. long.)

ural position, as though viewed from the side, and photographed together, as shown in figure 3. The characteristic orientation of the principal central spine and the comparative development of the accessory central spines are clearly illustrated. In h (*E. Bonkeræ*) the principal central spine is indistinguishable from the accessory centrals, and in d (*E. rectispinus*) the two accessory centrals resemble the radial spines.

A key to the species allied to *E. Fendleri* follows. This key includes the following three species, in addition to *E. rectispinus*, which have been described

since the monograph of Britton and Rose was published: *E. Boyce-Thompsoni*, *E. Bonkeræ*, and *E. Ledingii*.

E. Boyce-Thompsoni Orcutt, which stands between *E. Fendleri* and *E. rectispinus*, on the one hand, and *E. Engelmannii* (Parry) Rümpler, on the other, is very abundant throughout much of Gila County and extends into Graham and Pinal Counties. So far as known, the photograph in figure 2 is the first published illustration of this neglected species. A copy of the original description is appended in the belief that few students have access to files of Cactography.

E. Bonkeræ Thornber & Bonker has a more restricted range than *E. Boyce-Thompsoni* and probably is no more than a short-spined variety of that species. Two illustrations may be found, opposite pages 28 and 72, in *The Fantastic Clan* (MacMillan Co., 1932), where the original description appeared.

E. Ledingii Peebles, Cact. and Succ. Jour. 8, no. 3 (1936), has no close relative and occurs only on the slopes of Mt. Graham, in the Pinaleno Mts., Arizona. Adequate illustrations accompany the original description.

KEY TO PURPLE-FLOWERED ARIZONA SPECIES

Central spines wanting; areoles elliptic, the radial spines pectinate *E. rigidissimus* ⁵

Central spines present; areoles orbicular.

Central spines usually 2 to 6, or more, all well developed, more or less curved or twisted, the lower ones deflexed, commonly flattened and angled toward base; stems stout *E. Engelmanni*

Central spine solitary, terete, often accompanied by superposed accessory centrals, these much shorter and more or less like the radials.

Spines translucent, straw-colored, monochromatic; principal central curved near base, deflexed; accessory centrals 1 to 4, or wanting. *E. Ledingii*

Spines opaque, reddish-brown, dark-brown, whitened or ashy-gray, usually variegated.

Ribs 12 to 22, usually 14 to 18; principal central spine porrect or deflexed; 1 or 2 accessory centrals present on at least some areoles.

Spines 15 to 35 mm. long, the central sometimes longer; stems somewhat obscured by dense armament *E. Boyce-Thompsoni*

⁵ A related form with 4 or 5 short central spines has been collected recently in Cochise County, Arizona, by Wyatt W. Jones of Douglas.

Spines not more than 10 mm. long; stems not obscured *E. Bonkeræ*

Ribs 9 to 13, usually 8 to 10.

Centrals ordinarily curved and strongly ascending; except sometimes in age, flexible, 2.5 to 4.5 cm. long; accessory centrals wanting; radials straight or curved; stems rarely more than 10 or 12 cm. long, flaccid.

E. Fendleri

Centrals porrect; all spines straight; accessory centrals usually present on at least some areoles; stems rarely less than 15 cm. long, rigid.

Spines 1.0 to 2.6 cm. long, stout and rigid; stems few, usually 1 to 5 in number, 8 to 25 cm. long *E. rectispinus*

Spines 2.5 to 6.0 cm. long, relatively more slender and flexible; stems 5 to 15 in number, 25 to 45 cm. long.

E. rectispinus var. *robustus*

Echinocereus Boyce-Thompsoni Orcutt, Cactography, no. 3, part 1, 1926.—*Reprint of original description*: Plant caespitose, with the aspect of *E. Engelmanni*, few to a dozen or more heads from one root; stems 6–8 inches high, 2 in diameter; ribs 10–12, 10 mm. high, more or less tuberculate; radials 10, 8 mm. long, white, or often tipped with chocolate; central spines 1–2, 6–25 mm. long, erect, straight, terete; areoles ovate, 10 mm. apart, white-woolly when young, naked in age; flowers said to be variable in color, fruit unknown. Type locality: grounds of the Boyce Thompson Southwestern Arboretum, near Superior, Arizona, at an elevation of about 2300 feet.

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THE GENUS BLASTOSPORA ¹

E. B. Mains

BLASTOSPORA was described by Dietel in 1908. It was based on *Blastospora Smilacis*, which was described from a collection on *Smilax Sieboldi* obtained by T. Yoshinaga at Engyōji, Tosa, Japan, in October 1907. Dietel compares it to *Uromyces*. He emphasized the thin, hyaline walls of the teliospores, the absence of differentiate pores for the teliospores, and the separation of the promycelium from the empty germinated teliospore by a convex wall. Four additional species have been described in the genus, *B. Butleri* and *B. Hygrophilæ* by the Sydows and Butler in 1912, *B. Itoana* by Togashi and Onuma in 1931, and *B. ascotela* by Sydow and Mitter in 1935.

A study ² of this genus has resulted in the discovery of an interesting situation. The type speci-

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Paper from the Department of Botany and Herbarium of the University of Michigan.

² The writer is indebted to Dr. G. Samuelsson of the Naturhistoriska Riksmuseet, Sweden, to Dr. N. Hiratsuka

men of *Blastospora Smilacis* bears minute telia. These do not develop within the tissue of the host but on the exterior. The mycelium emerges through the stomata and the spore-producing cells develop outside. These form a compact pulvinate mass, 50–60 μ in diameter. From them, the teliospores arise. These are globoid, with very thin hyaline walls. They germinate at once with cylindrical basidia. Apparently the wall of the basidium is not a continuation of the wall of the teliospore, as stated by Dietel. Several germinating teliospores were seen in which the protoplasm had contracted somewhat, showing a clear separation from the wall of the teliospore and the young basidium projecting through an apical opening in it. The wall of the basidium is as thick as that of the teliospore, and the difference usually of Tottori Agriculture College, Japan, and Dr. D. H. Linder of the Farlow Herbarium, Harvard University for a number of the collections studied in this investigation.

is not very evident. The telia often occur in groups, and specially after the teliospores have started to germinate, they form fairly large pulverulent masses.

The situation in regard to the uredinia is puzzling. In some collections urediniospores are produced in the telia. These are globose or broadly ellipsoid, $20-25 \times 24-28 \mu$ and coarsely echinulate. The type specimen also bears uredinia which differ markedly from the telia. They are subepidermal and rupture the epidermis. The urediniospores are larger ($16-22 \times 23-32 \mu$) than those from the telia and less prominently echinulate.

Blastospora Itoana has also been described from *Smilax* in Japan. The specimens of this species which have been available for study have telia similar to those of *B. Smilacis*, the teliospores being somewhat smaller. Only teliospores have been described for this species. In this study, uredinia have been found on several collections. These resemble the telia. The mycelium emerges through the stomata and develops compact masses of spore-producing cells on the surface of the leaf. The question therefore arises whether or not the subepidermal uredinia occurring on the type of *Blastospora Smilacis* really belong to that species.

A study of type specimens of the other species of *Blastospora* has resulted in the conclusion that these do not belong in the genus. *Blastospora Butleri* was described from a collection on *Jasminum malabaricum* made by S. L. Ajrekar at Matheran, Bombay India, Nov. 15, 1911. It has subepidermal telia. The teliospores are cylindric or clavate, sessile, thin-walled, hyaline, arising in groups of 5-9 from basal cells. This is evidently a species of *Chaonia*, and the combination *Chaonia Butleri* (Syd.) nov. comb. is proposed.

Blastospora Hygrophilae was described from a collection on *Hygrophila salicifolia* made by R. Sen at Chittagong, India, Sept. 11, 1911. The telia are subepidermal. The teliospores are broadly ellipsoid, thin-walled, hyaline, pedicellate, arising from a compact sporogenous layer. This is evidently a species of *Maravalia*, and the combination, *Maravalia Hygrophilae* (Syd. & Butler) nov. comb. is proposed.

Blastospora ascotela was described from a collection on *Hedyotis stylosa* made by J. H. Mitter (107), Ootacamund, British East India, Oct. 6, 1932. The telia are subepidermal, and the teliospores are cylindric, thin-walled, hyaline, pedicellate, arising from a compact sporogenous layer. This is evidently a species of *Maravalia*, and the combination *Maravalia ascotela* (Syd.) nov. comb. is proposed.

Dietel (1928) has placed *Blastospora* in the tribe Eriosporangieae of the Pucciniaceae. The manner of development of the telia excludes it from this tribe. In Dietel's classification it clearly belongs in the Hemileieae. It differs from *Hemileia* and *Gervasia* in that the sporogenous cells develop outside the host. In *Hemileia* the basal cells develop within the host, and the pedicels of the teliospores project through the stoma. It is nearer *Gervasia*. *Gervasia* was de-

scribed by Raciborski (1909) from a rust of *Rubus*, *C. Rubi*, from Java. Apparently this is known only from the original collection which has not been available for this study. Raciborski's description of the telia indicates important differences. He states that the teliospores are borne on a cell which projects through the stoma. This cell swells outside of the stoma, and from the swelling 4-15 teliospores develop. The teliospores are globose and pedicellate.

Blastospora Dietel, Ann. Mycol. 6: 222. 1908.—Uredinia superstomatal (possibly also subepidermal), spore-bearing cells forming a small compact group above the stoma from mycelium emerging from it; urediniospores obovoid or ellipsoid, echinulate, pedicellate.

Telia superstomatal, spore-bearing cells forming a small compact group above the stoma from mycelium emerging from it; teliospores globose or ovoid, the wall very thin, hyaline, pedicellate, germinating at once.

Type species: *Blastospora Smilacis* Dietel.

Blastospora Smilacis Dietel, Ann. Mycol. 6: 223. 1908.—Uredinia associated with the telia (connection doubtful), subepidermal, the urediniospores broadly obovoid or ellipsoid, $16-22 \times 22-32 \mu$, the wall yellowish, $1.5-2 \mu$, moderately echinulate; urediniospores in the telia subglobose or broadly ellipsoid, $20-25 \times 24-28 \mu$, the wall yellowish, $1.5-2 \mu$, coarsely echinulate, the pores obscure.

Telia hypophyllous, superstomatal from hyphae emerging through the stomata, spore-bearing cells forming a compact lenticular mass above the stomata, often crowded into groups $1-2$ mm. across, soon pulverulent from germination; teliospores ovoid or subglobose, $26-36 \times 40-55 \mu$, the wall hyaline, very thin, 1μ or less, pedicellate, the pedicels $6-12 \times 16-32 \mu$, germinating immediately through an apical opening, producing basidia $20-26 \times 90-100 \mu$.

Specimens examined: *Smilax Sieboldi* Miq. Engyōji, Mikazuki-mura, prov. Tosa, Japan, Oct. 1907, T. Yoshinaga, type; Hashiratani, Jūroku-mura, prov. Tosa, Oct. 31, 1909, T. Yoshinaga; Mt. Iwayama, pref. Iwate, Oct. 30, 1932, K. Togashi.

Subepidermal uredinia were found on two specimens, the type and Yoshinaga's collection of Oct. 31, 1909. They differ considerably in their development from the telia and from the uredinia of the following species, and the question arises whether or not they belong to this species. However, no other species on *Smilax* in Japan has been reported having uredinia which resemble them.

Blastospora Itoana Togashi and Onuma, Bot. Mag. 45: 6. 1931.—Uredinia minute, scattered in discolored areas, superstomatal from hyphae emerging through the stomata, the spore-bearing cells forming a compact mass above the stomata; urediniospores globose or subglobose, $16-20 \times 16-22 \mu$, the wall yellowish, $1.5-2 \mu$ moderately echinulate, the pores obscure.

Telia minute, scattered in discolored areas, superstomatal from hyphae emerging through the stomata,

the spore-bearing cells forming a compact mass above the stomata; teliospores globoid or ovoid, 24-38 \times 30-44 μ , the wall hyaline, thin, 1 μ or less, pedicellate, germinating at once.

Specimens examined: *Smilax Oldhami* Miq. Mt. Daisen, prov. Hôki, Japan, Aug. 20, 1930, Naohide Hiratsuka; Aug. 24, 1931, Naohide Hiratsuka; Skinkeiji (Soto-kongô) prov. Kôngen, Korea, Aug. 24, 1934, Naohide Hiratsuka.

Uredinia were found on all the specimens, most abundantly on the collection of Aug. 24, 1934.

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THE REACTIONS OF MYXOMYCETOUS SWARM-CELLS TO TEMPERATURE¹

Robert F. Smart

TEMPERATURE, accepted as an environmental factor generally influential in protoplasmic activity, has been shown by de Bary (1884), Constantineau (1906), and the writer (Smart, 1937), to exert a definite influence upon the germination of myxomycetous spores. In so far as the writer is aware, however, the literature reveals no attempt on the part of students of this interesting group of organisms to study the influence

ample, it was evident that the swarm-cells of most species studied were able to continue their activity at temperatures much higher than those at which the spores of the same species had been able to germinate.

As the influence of temperature upon the behavior of active protoplasm is of considerable biologic interest in itself, the investigation of its influence upon the simple, presumably primitive, naked swarm-cells

TABLE 1. Reactions of the swarm-cells of *Fuligo septica* to high temperatures.

Trial	Temperatures			
	Quiescence	1st. motion	Flagellate motion	Normal activity
1	41°C.	38°C.	36°C.	33.5°C.
2	40°C.	38°C.	35°C.	32°C.
3	41.5°C.	37°C.	34.5°C.	33°C.
4	39.5°C.	38°C.	35°C.	31°C.
5	41°C.	37°C.	34°C.	32°C.
6	40°C.	38°C.	35°C.	33°C.
7	39°C.	37.5°C.	34°C.	31°C.
8	41°C.	39°C.	35°C.	33°C.
9	41.5°C.	39°C.	36°C.	32.5°C.
10	40°C.	37°C.	34°C.	33°C.

of temperature upon the behavior of the flagellate swarm-cells derived from the spores of representative species. The writer (1937), while studying the influence of temperature upon spore germination, was impressed by the reaction of the swarm-cells to the temperatures used in those experiments. For ex-

which typically initiate the life cycle in the Myxomycetes is of distinct significance.

It seemed desirable, therefore, to investigate this point to determine whether or not the various species show specific differences in their behavior. Accordingly a series of preliminary experiments were first performed upon the swarm-cells of species such as *Fuligo septica*, *Enteridium rozeanum*, and *Reticularia lycoperdon* selected because of their high percentage of germination and normally long swarming period (usually 4 days or more). The work was then extended to a larger and more representative number of species (cf. table 2). The experiments were carried on both in hanging drop cultures in electrically

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controlled micro-slide incubators manufactured by the Chicago Surgical Supply Company and in Syracuse glass cultures in a modification of the micro-stage incubators described by Cotner (1930). The medium previously found most favorable for the activity of the swarm-cells was used in each case (Smart, 1938). After the spores had germinated and the swarm-cells had developed, the temperature of each incubator was gradually raised by 1° intervals, at each of which it was maintained for ten minutes to permit the swarm-cells to become accustomed to the higher temperatures and to facilitate observation.

giving the reaction of the swarm-cells of *Fuligo septica* to high temperatures.

The results shown in table 1 reveal certain points of interest: (1) the temperature at which the swarm-cells of *Fuligo septica* round up and become quiescent ranges from 39° to 41.5°C. and (2) the optimum temperature for the activity of the swarm-cells of this species is from 31° to 33.5°C.

The results obtained from studying the reaction of the swarm-cells of representative species of Myxomycetes to high temperatures are summarized in table 2.

TABLE 2. Reaction of myxomycetous swarm-cells to high temperatures. Nomenclature is that used in Macbride and Martin (1934).

Species	Temperatures			
	Quiescence	1st. motion	Flagellate motion	Normal activity
<i>Fuligo septica</i>	39°-41.5°C.	37°-39°C.	34°-36°C.	31°-33.5°C.
<i>Badhamia utricularis</i>	35°-38°C.	32°-34°C.	30°-32°C.	28°-30°C.
<i>Badhamia rubiginosa</i>	39.5°-42°C.	38°-39°C.	35°-36°C.	32°-34°C.
<i>Physarum didermoides</i>	40°-43°C.	38°-39°C.	35°-36°C.	32°-34°C.
<i>Physarum cinereum</i>	38.5°-42°C.	37°-40°C.	34°-35°C.	32°-33°C.
<i>Didymium melanospermum</i> ..	35°-38°C.	33°-34°C.	31°-33°C.	29°-30°C.
<i>Craterium leucocephalum</i> ...	36°-37°C.	34°-35°C.	32°-33°C.	27°-29°C.
<i>Physarella oblonga</i>	39°-41°C.	36°-37°C.	33°-35°C.	30°-31°C.
<i>Diachea leucopodia</i>	34°-36.5°C.	32.5°-34°C.	30°-31°C.	26°-28°C.
<i>Stemonitis fusa</i>	37°-39°C.	33°-34°C.	30°-31°C.	27°-29°C.
<i>Stemonitis splendens</i>	39°-41°C.	36°-38°C.	33°-35°C.	29°-31°C.
<i>Conatricha nigra</i>	40°-42°C.	37°-39°C.	34.5°-37°C.	31°-33°C.
<i>Lamproderma arcyrionema</i> ..	39.5°-41°C.	36°-38.5°C.	33°-35°C.	31°-32°C.
<i>Cribraria intricata</i>	38.5°-40°C.	37.5°-38°C.	34°-37°C.	29°-32°C.
<i>Cribraria aurantiaca</i>	40°-42°C.	36°-39°C.	33°-36°C.	28°-30°C.
<i>Didymium cancellatum</i>	38°-40°C.	37°-38°C.	33°-35°C.	30°-32°C.
<i>Enteridium roseanum</i>	39°-42°C.	38°-39°C.	34°-36°C.	27°-30°C.
<i>Reticularia lycopodon</i>	38°-39°C.	35°-37°C.	32°-35°C.	26°-30°C.
<i>Didydiaethalium plumbum</i> ..	40°-42°C.	38°-40°C.	34°-40°C.	30°-33°C.
<i>Lycogala epidendrum</i>	39°-43°C.	36°-38°C.	33°-35°C.	29°-32°C.
<i>Perichaena depressa</i>	41°-43°C.	38°-40°C.	34°-36°C.	31°-33.5°C.
<i>Arcyria cinerea</i>	39°-41°C.	37°-38°C.	33°-35°C.	28°-31°C.
<i>Arcyria denudata</i>	40°-43°C.	37°-39°C.	34°-35°C.	30°-33°C.
<i>Hemitrichia clavata</i>	37°-40°C.	34°-37°C.	32°-33°C.	29°-31°C.

The point at which the swarm-cells became quiescent was noted; the temperature was then lowered by 1° intervals, and the point at which the hitherto quiescent protoplast showed signs of any resumption of movement was recorded, and, finally, record was made of the temperature at which normal activity of the swarm-cells ensued. The culture was maintained at this optimum for one hour, after which the temperature was again raised, and the points at which changes in the activity of the swarm-cells occurred were recorded. This procedure was repeated until the swarm-period finally terminated either in the encystment of the swarm-cells or the production of zygotes or myxamoebae which failed to return to the flagellate stage. In general the manner of recording observations is more readily exhibited by a sample protocol (table 1),

From table 2 it can be seen that temperatures at which quiescence of the swarm-cells occurs varies with the species. For example, in *Physarum didermoides* the swarm-cells become quiescent when the temperature reaches a point between 40° and 43°C., while the swarm-cells of *Diachea leucopodia* become quiescent at 34° to 36.5°C. Likewise, the optimum temperature for the activity of the swarm-cells varies with the species, for in *Badhamia rubiginosa* normal activity of the swarm-cells ensues at 32° to 34°C., while in *Diachea leucopodia* normal activity occurs at 26° to 28°C. In general, however, the temperature at which normal activity occurs bears a direct relation to the temperature at which quiescence occurs. For example, if the temperature at which quiescence of the swarm-cells occurs is high in the case of a

given species such as *Fuligo septica*, the temperature at which normal activity of the swarm-cells of the same species ensues will in like manner be comparatively high.

In their responses to changes in the temperature, the swarm-cells exhibited some rather striking reactions which cannot be shown in the table. For example, as the temperature approached the maximum at which complete quiescence occurred, the swarmers were found to stop abruptly in their forward path and rotate about their short axes for several minutes, after which they would swim away in some direction other than that formerly taken. These periods of spasmodic rotations became more frequent as the temperature rose, until finally the swarm-cell assumed the undulatory, creeping stage, during which occurred vigorous protoplasmic streaming accompanied by continuous change of form. As the temperature approached that at which quiescence would occur, the swarm-cells became ever more rounded in outline and gradually retracted the flagella. If the temperature was held at this point for periods varying in the different species from thirty minutes to one hour, the swarm-cells would round up and form microcysts. If, however, the temperature was lowered gradually, the protoplasm of the quiescent swarm-cells began to move and put out pseudopodia, creeping about as myxamoebae. Finally a flagellum was developed for each swarm-cell, and after a brief undulatory stage, the swarm-cell would jerk its body into its characteristic comma-shape and swim off.

In addition to the above responses of the swarm-cells to temperature the writer observed that in *Fuligo septica*, *Badhamia utricularis*, *Physarum didermoides*, *Physarum cinereum*, *Didymium melanosperrum*, *Physarella oblonga*, *Stemonitis fusca*, *Enteridium rozeanum*, *Reticularia lycoperdon*, *Dictydiaethalum plumbeum*, and *Arcyria denudata* the fusion of the swarm-cells in the cultures at temperatures between 30° and 33°C. seemed more abundant than at any other temperatures in the range of these experiments or than in the control cultures kept at room temperature. An account of further experimentation in a study of this point will be presented in a subsequent paper.

Having observed the effects of high temperatures upon the behavior of the swarm-cells, the next logical step seemed to the writer to be a study of the responses of the swarm-cells to low temperatures. Toward this end a series of experiments were performed.

To determine the effects of low temperatures upon the swarm-cells, an electrically controlled refrigerator was used for establishing and maintaining temperatures below 7°C. It should be noted, however, that even though the temperature was thus accurately controlled, sudden changes necessarily took place when the cultures were removed to room temperature for examination.

The swarm-cells of all the species tested, when subjected to temperatures as low as 7°C., remained active for more than half of the normal swarming

period typical of room temperature, and some species, such as *Fuligo septica*, *Enteridium rozeanum*, *Arcyria denudata*, and *Hemitrichia vesparium*, withstood temperatures as low as 2°C. without encysting immediately.

The case of *Fuligo septica* is especially worthy of comment. The writer found that if the spores of this species were allowed to germinate at room temperature and the culture of active swarm-cells then transferred to the refrigerator at 4°C., the swarm-cells became sluggish and only rarely swam from place to place, usually remaining in one position, and by a slow vibration of the flagellum rocking back and forth on their long axes. No fusions occurred, no microcysts were evident, no bacteria developed in the culture, and the swarm-cells could be maintained unchanged in this state for periods as long as four weeks and more. Upon removing the culture from the refrigerator and again raising the temperature to the optimum, the swarm-cells became most active and swam rapidly through the medium in characteristic rotating fashion.

It is clear from the foregoing that in a large number of Myxomycetes a temperature range between 27° and 34°C. is most favorable for the continuation of the normal activities of the swarm-cells. It is also clear, however, that the swarmers can retain their vitality and identity over a relatively very wide range of temperatures. The latter point especially has not been brought out in previous work and definitely changes our views as to the adaptability of this phase in the Myxomycete life cycle. Furthermore this adaptability probably has actual survival value under natural conditions in the field, since the swarm-cells, being merely slowed down in their activities by reduced temperatures, are not induced to form resistant microcysts which must develop into swarmers again but rather retain their identity as swarmers through temporary fluctuations of temperature, thus eliminating the delay which would be necessary for the germination of the microcyst upon the return of favorable temperatures.

SUMMARY

Twenty-four species representative of the more common groups of endosporeous Myxomycetes were studied in an effort to determine the influence of temperature upon the activity of the flagellate swarm-cells.

When subjected to temperatures above room temperatures, the temperatures at which quiescence of the swarm-cells occurs was found to vary with the species. For example, in *Physarum didermoides* quiescence of the swarm-cells occurs between 40° and 43°C., while in *Diachea leucopodia* the swarm-cells become quiescent between 34° and 37°C. Likewise, the optimum temperature for the activity of the swarm-cells varies with the species, for in *Badhamia rubiginosa* normal activity occurs at 26° to 28°C. In general, however, if the temperature at which quiescence of the swarmers of a given species occurs is high, the temperature at which normal activity of

the swarm-cells of the same species ensues is in like manner comparatively high. The optimum temperature range for most species studied is from 27° to 34°C.

When subjected to temperatures below room temperatures, all species studied are slowed down in their activities, and some species such as *Fuligo septica*, *Enteridium rozeanum*, *Arcyria denudata*, and *Hemi-*

trichia vesparium withstand temperatures as low as 2°C. without encysting immediately. Upon removing the cultures from the refrigerator and restoring the optimum temperatures, the swarm-cells become active and swim rapidly through the medium in their characteristic rotating fashion.

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THE MORPHOLOGY OF THE BASIDIUM¹

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THE STRICT homology between ascus and basidium and between the ascomycenous hyphae of the Ascomycetes and the dicarcon mycelium of the Basidiomycetes may be regarded as completely established. There remains, however, a tendency to think of the basidium in terms of the clavate, homobasidial type and to regard the less stabilized basidia of the Heterobasidiomycetes as in some way irregular or aberrant, to be interpreted in terms of the morphologically simpler clavate structure, with its four sterigmata arising from the apex of the scarcely modified gonotocant. Thus, even in some recent papers, the thick cylindrical tubes which arise from the gonotocant in such forms as the Tremellaceae and the Dacrymycetaceae are still referred to as "sterigmata," in spite of the fact that over forty years ago Dangeard (1895) clearly pointed out the distinction between the "tube germinatif" and the true sterigma at its tip in *Tremella mesenterica*. The most fruitful study bearing on this point is that of Neuhoff (1924), who introduced the terms hypobasidium and epibasidium in connection with the heterobasidium. As used by Neuhoff, a hypobasidium is that cell in which nuclear fusion takes place (i.e., the zeugite in Gäumann's sense), whether it undergoes a resting stage or not, but which does not produce sterigmata directly. The term corresponds in part with the probasidium of Van Tieghem (1893), but is more inclusive, and replaces it in the rusts and smuts. The epibasidium is an outgrowth from the hypobasidium serving either singly or in connection with others to carry protoplasm and nuclei through the frequently gelatinous

matrix, in which the hymenium is imbedded, to or beyond the surface, where true sterigmata are developed and the spores are borne.

Donk (1931, p. 78-81) rejects Neuhoff's terminology and defines the probasidium as "that part or stage of the basidium in which karyogamy takes place," coining the new term metabasidium for "that part or stage in which the diploid nucleus divides." According to this conception, the probasidium merges into the metabasidium in all Basidiomycetes except the rusts and smuts and those members of the Auriculariaceae in which there is a basal vesicle. Such a treatment completely ignores the problems raised by the epibasidia of the great majority of the Heterobasidiomycetes.

With certain qualifications, Rogers (1934), in his discussion of basidial morphology, adopts Neuhoff's terminology, and during the past decade Neuhoff's terms have been usefully employed in a number of papers dealing with the Heterobasidiomycetes. Recently, however, doubts have been expressed as to the validity of the concepts implied by them.

Boedijn (1937) starts with the premise that the use of the terms hypobasidium and epibasidium assumes that both structures "are parts of the basidium and not separate organs." This premise is obviously correct. He then proceeds to give reasons why he regards the assumption as unjustified. In a species of *Helicogloea* (*H. indica*) which he describes, the vesicular basal structure, for which he uses the term probasidium in its earlier and restricted sense, is separated from the enlarged, septate, spore-bearing extremity by a long, slender, connecting portion. To

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Boedijn, this middle attenuation seems sufficient justification for regarding the basal and distal portions as separate organs. The situation in *H. indica*, as he illustrates it, is indeed striking, but there is no reason for interpreting it as anything more than an extreme development of a tendency already well established in other species of *Helicogloea*. In her study of *H. Lagerheimii*, Baker (1936) shows basidia in which the basal structure is separated from the distal portion by a slender connecting tube (pl. 7, fig. 12, 13) not, to be sure, so extraordinarily long as in Boedijn's species, but wholly comparable, and finds other basidia (pl. 8, fig. 19, left) with a mere constriction as in *H. intermedia* (Linder, 1929, pl. 41, fig. 17). Furthermore, the variation in *H. intermedia* is such as to establish the fundamental homology of the *Helicogloea* basidium with that of *Jola* and, through *Jola*, with the other members of the Auriculariaceae. It seems clear that Boedijn's contention that the two parts of the basidium in *H. indica* are separate organs cannot be admitted.

Further objection is made on the ground that while in the Auriculariaceae the hypobasidium is 1-celled and the single epibasidium 4-celled, in the Tremellaceae the hypobasidium is 4-celled, and there are four 1-celled epibasidia. Aside from the fact that this is incorrect, since the epibasidia in the *Tremella* type of basidium are not separate cells but extensions of the hypobasidial segments, it is not clear why this should be regarded as invalidating Neuhoﬀ's contentions. In both cases, the effect is to convey the nuclei and the accompanying cytoplasm toward the place where the spores are formed. The formation of septa is linked with another process, meiosis, and follows it at once. The tubular appendages are not, as Boedijn states, a means of elevating the spores to the surface. In the waxy members of the Tulasnellaceae (*Tulasnella*) the basidia are already at the surface, and the epibasidia, although clearly distinguished, are short and thick. This condition is approached in certain of the Tremellaceae—e.g., in some of the thin and waxy species of *Sebacina*. In the Dacrymycetaceae, as in the majority of the Auriculariaceae and nearly all the Tremellaceae, the spores are not elevated; the epibasidia grow to the surface, the sterigmata are protruded, and then only are the spores formed and matured in their first and final position.

That all Heterobasidiomycetes possess basidia which become clearly differentiated into hypobasidia and epibasidia is no part of the claim of those who would defend the essential validity of Neuhoﬀ's concepts. In *Ecchyna*, the practically sessile spores are borne upon the segments of the scarcely modified hypobasidium. In *Hyaloria*, the only angiocarpous genus with tremellaceous basidia, the distinction between hypobasidium and epibasidium holds, but the sterigmata are suppressed (Martin, 1937, fig. 16, 19). In *Sirobasidium*, as Boedijn points out, both epibasidia and sterigmata are suppressed, but this does not prove their non-existence in other forms.

Lohwag (1937) attacks Neuhoﬀ's contentions on cytological grounds. He regards all outgrowths from basidia as germination hyphae ("Keimschläuche") and divides them into three groups: those of the first order, in which the nuclear content is diploid; those of the second order, formed after meiosis, into which the monoploid nuclei pass without noteworthy modification; and those of the third order, the greatly constricted sterigmata, through which the nuclei pass only with marked modification of their shape and staining reactions. Lohwag (fig. 12, a and b) illustrates in schematic fashion a typical tremellaceous basidium and one of the auriculariaceous type arising from a thick-walled gonotocent. In such forms, the latter structure exhibits germ-tubes of all three orders, while the former lacks those of the first order. With the strict logic of such a presentation there can be no quarrel. It is inadequate, however, for two reasons. First, organisms as found in nature rarely follow the rules of logic, and attempts to fit them into too rigid a frame have always resulted in artificial systems. Second, the basidium is, to be sure, a germinating gonotocent, but it is also more than that. It is, as abundantly demonstrated by Buller (1909), a highly specialized organ for the production and violent discharge of spores. In order to be violently discharged, basidiospores must be borne in the air. The means of transferring the monoploid nuclei to a subaerial position are varied, particularly in those presumably lower groups where the fructification is commonly gelatinous, but fundamentally these are all variations of a single basic mechanism. This mechanism involves both morphological and cytological features, and these must be interpreted together. If, in the past, morphological facts, because of their greater prominence, have been unduly emphasized, it is still true that they are facts, and to disregard them for a purely cytological explanation is to lead to such absurdities as the wholly unwarranted emphasis on sticho- and chiasmobasidia, which both Rogers and Lohwag justifiably condemn.

Every fruitful concept is subject to modification; Neuhoﬀ's interpretation of the basidium is no exception. In my opinion, it has been extraordinarily useful in the recent development of our understanding of the nature and significance of this extremely variable but basically constant organ. The very objections which are being urged against it at the present time have to a considerable degree been the result of investigations to which it has furnished a stimulus. If it can be reformulated in the light of new information, it is still capable of serving better than any other suggested interpretation to clarify our concept of the basidium. The following paragraphs attempt such a restatement.

✓ Although the basidium and the asexual are homologous, a typical basidium differs from a typical asexual in that the tetrads or their daughter nuclei do not become differentiated into spores lying free within the gonotocent wall but pass into projections of the wall, these projections, or more commonly, portions

of them, being separated as the outer walls of the basidiospores. A basidium, then, may be defined as a cell in which two nuclei fuse, preliminary to meiosis, following which, directly or indirectly, spores are formed in extensions of the gonotocont wall. Spore formation may or may not be preceded by septation of the basidium. If septation occurs, a mature basidium may be 2- to 4-celled, or occasionally 5-, 6-, or 7-celled (Rogers, 1933, fig. 13a). The term basidium may properly be extended to structures in which fusion and reduction do not take place, when they are obviously morphologically homologous with the karyallage types.

The clavate basidium characteristic of most hymenomycetes, with usually four sterigmata borne directly on the summit of the gonotocont, is a reduced, not a primitive type. In many gasteromycetes, and in a few of the lower forms, this reduction has been carried farther by the elimination of sterigmata.

The primitive basidium may be postulated to have been one in which the tetracytes entered into extensions of the gonotocont wall, from which they were violently shot off, as are many ascospores, and by a modification of the same mechanism, applied, however, to the individual spores instead of to the spore-group as a whole; or, as suggested by Vuillemin (1893) and by Rogers (1932), it may have been a form in which the ascus germinated by the production of conidia. Either view obviously favors a phragmobasidium as the primitive type rather than a holobasidium, but does not completely exclude the latter. Rogers would choose the basidium of *Tulasnella* as perhaps the best illustration of a primitive basidium in living fungi. The fact that the basidial extensions may be separated by abstriction before germination in the same genus (Martin, 1931, p. 7) merely illustrates the flexibility of such a primitive type. The externally similar basidia and spores of certain gasteromycetes are to be regarded not as primitive, but as reduced types, first, because closely related forms may have basidia with sessile spores or with typical or modified sterigmata, and second, because no gasteromycete spores germinate by repetition.²

Meiosis may take place either within the limits of the original cell wall, which in such case is enlarged, or, especially when the zeugite is thick-walled, as in the rusts and smuts and some species of *Septobasidium*, in an extension of the cell wall. The place of meiosis should not be regarded as a fundamental basidial character but as a secondary adaptation, conditioned, at least in some instances, by the nature of the zeugite.

The term "probasidium," first proposed by Van Tieghem (1893) for the thick-walled resting zeugite (teliospore) of the rusts and smuts, may properly and conveniently be extended, as suggested by Donk (1931), to all basidia in the same stage—that is, from the time the zeugite is formed until it or the gonotocont begins to develop one or more protuberances,

whether these protuberances are to become epibasidia or sterigmata or are to be transformed directly into basidiospores.

The term "hypobasidium" may be applied to the original cell from the time the protuberances begin to appear until the final discharge of the spores and the collapse of the entire structure. It follows that the clavate basidium of an agaric, in its fully developed stage, consists of hypobasidium, sterigmata, and spores; that of a *Cyathus* or *Phallus*, of hypobasidium and spores only. It is obvious that the term has no special application to these forms and need be used in connection with them only when it is desired to emphasize morphological homology. The hypobasidium may be emptied of protoplasm and cut off from the epibasidia, as in *Tulasnella*; it may be partially emptied, but not systematically cut off, as in the Daermycetaceae; it may be divided into four compartments by three septa (not two, as Lohweg states), as in *Tremella*; or it may be merged with the epibasidium, as in *Auricularia*.

The term "epibasidium" may be applied to any structure which intervenes between the hypobasidium and a sterigma, or, rarely, as in *Hyaloria*, an asterigmate spore. It may be simple and continuous with the hypobasidium or its segments, as in *Tremella* and the Daermycetaceae, or simple, but cut off from the hypobasidium, as in *Tulasnella*, or compound, as in *Helicogloea* and the rusts. In *Helicogloea*, if the epibasidia are immersed, each cell may develop a lateral extension. The same thing is true in rusts with gelatinous telia, such as *Gymnosporangium*. That this variation represents no more than an ecological adaptation may be demonstrated by the fact that epibasidia which reach the surface and then bend over so that all cells are exposed, as they frequently do when the fructification is comparatively dry, form sterigmata directly, whereas when the basidiocarp is wetter, the lateral extension intervenes. All gradations may sometimes be observed on the same fructification.

The term "sterigma" is to be used only for a narrowed pedicel upon which a basidiospore is borne and from which it is violently discharged or to an obvious modification of such a structure, such as is found in a number of gasteromycetes. It is on no account to be applied to the stalk upon which a conidium or a sporangium is borne. In the case of basidiospores germinating by repetition, the products of germination are secondary basidiospores; hence the tube issuing from the original spore is a secondary epibasidium and is tipped by a secondary sterigma.

It is contended that the definitions of the terms basidium, probasidium, hypobasidium, epibasidium, and sterigma, as here stated, represent a legitimate extension and clarification of the original meanings in accordance with present-day ideas as to the nature of the basidium and that when employed as here defined, they serve to embody useful and significant concepts.

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² See Rogers, 1934, p. 163, for more complete discussion of this point.

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THE PHYCOMYCES ASSAY FOR THIAMIN (VITAMIN B₁): THE METHOD AND ITS CHEMICAL SPECIFICITY¹

James Bonner and James Erickson

THE IDEA that fungi may require vitamin-like special growth factors in addition to the ordinary nutrient substances has been expressed repeatedly since the work of Wildiers (1901) upon the "bios" substances which are essential to the growth of yeast. It is now well known that many fungi do indeed require such growth factors. This was shown particularly well by the work of Schopfer (1934) and Burgeff (1934), who found that *Phycomyces Blakesleeanus* must have an external supply of thiamin (vitamin B₁); and this fact has been expanded by Schopfer and Jung (1937) into a quantitative biological assay for the vitamin.

A knowledge of the chemical specificity of this assay is desirable for several reasons. It is in the first place necessary to know what substances are capable of replacing the vitamin as a growth factor for *Phycomyces*, in order to establish the validity of the test as applied to the thiamin content of plant materials.

In the second place, it should be possible to contribute to the understanding of how the vitamin acts as a growth factor for *Phycomyces* by determining which groups of the vitamin molecule are essential and which are non-essential to this activity.

MATERIALS AND METHODS.—The basic thiamin-free nutrient medium used for these experiments is that described by Schopfer and Jung (1937) and has the composition shown in table 1. Salts and dextrose of the usual C.P. quality were found to give satisfactory results and to be free from significant amounts of

thiamin. Commercial asparagin, however, was found to contain, as an impurity, either thiamin or a related substance (see below) having growth activity upon *Phycomyces*. This impurity could be largely removed by three recrystallizations of the asparagin from water.

TABLE 1. *Composition of the basic medium for the growth of Phycomyces Blakesleeanus.*

MgSO ₄ ·7H ₂ O	0.5 gms.
KH ₂ PO ₄	1.5 "
Asparagin	4.0 "
Dextrose	100.0 "
Dist. water	1 liter

Cultures were made with 10 cc. of the nutrient medium in 50 cc. Pyrex Erlenmeyer flasks. This amount of medium is smaller than that recommended by Schopfer (25 cc. in 100 cc. flasks), but the resultant saving of materials is considerable, and there is no corresponding decrease in the accuracy of the determinations. To the 10 cc. of nutrient medium was added 0.1 cc. of the particular solution to be tested for growth-promoting activity. The culture flasks were then autoclaved for 15 minutes at a pressure of 15 pounds.

Stock cultures of *Phycomyces* were kept upon malt agar (2 per cent malt in 2 per cent agar). The culture vessels were inoculated with uniform volumes of a spore suspension prepared from these stock cultures. The number of spores used as an inoculum was found to exert no significant effect, over a 1000-fold range, either upon the growth of control cultures in thiamin-free medium or upon the growth of the mold in medium containing large amounts of this substance. After the culture, vessels had been inocu-

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lated, they were allowed to remain for ten days in an incubator at 25°C. As has been shown by Schopfer (1937), ten days suffices for the mold to attain its maximum growth under these conditions. At the end of this period the mycelium was filtered from each flask onto separate weighed filter papers, washed, dried at 60°C. for 24 hours, and weighed. In a single experiment, 50 to 100 culture flasks were used, and each concentration of each substance was tested in duplicate. The values presented in the tables below, however, are based upon 8 or more determinations.

TABLE 2. Dry weight of mycelium as a function of growth factor concentration.

Growth factor conc.: mols/liter	Dry weight of mycelium: mgs.				
	10 ⁻⁶	10 ⁻⁷	0.5 × 10 ⁻⁷	0.25 × 10 ⁻⁷	0.125 × 10 ⁻⁷
Thiamin	101	71	56	29	15
Pyrimidine + thiazole * ..	103	66	56	28	12

* The pyrimidine and thiazole whose structures are shown in figure 1.

ACTIVITY OF THIAMIN AND ITS COMPONENT PARTS.—In table 2 is shown the relation between amount of thiamin in a single culture flask and the dry weight of *Phycomyces* mycelium produced. The amount of mycelium produced under these conditions is approximately proportional to the amount of available thiamin for concentrations of this substance up to approximately 0.5×10^{-7} molal (0.5×10^{-9} mols per culture flask). Amounts of thiamin larger than this permit of still larger dry weights of mycelium, but the response is not linear. A second factor must therefore become limiting in the presence of large amounts of thiamin. This second factor, as Schopfer (1937) has shown, and as confirmed in the present work, is the amount of asparagin in the medium. If more asparagin is used in the basic medium, the response to added thiamin is linear over a wider range of growth factor concentration, and the maximum weights of mycelium are increased. The advantages which this might have, however, are offset by the greater amount of asparagin which must be used. For the determination of the growth promoting effect of an unknown substance, it is then necessary to adjust the amount of the substance so that the weight of mycelium will be small—i.e., will fall within the linear range of response.

The structure of the thiamin molecule is reproduced in figure 1. It is composed of two parts: (1) a substituted thiazole nucleus, and (2) a substituted pyrimidine nucleus which is linked through a methylene bridge to the thiazole. It has been shown that several organisms, including among others *Phycomyces* (Schopfer and Jung, 1937b; Robbins and Kavanagh, 1937; Sinclair, 1937), *Staphylococcus aureus* (Knight, 1937), the flagellate *Polytoma coeca* (Lwoff and Dust, 1937), and the isolated pea root (Bonner, 1938), are able to utilize a mixture of these two portions (B and C in fig. 1) as a substitute for the vitamin. In table 2 it is shown that the activity

of an equimolecular mixture of the vitamin pyrimidine and the vitamin thiazole is quantitatively essentially identical with that of the vitamin itself in promoting the growth of *Phycomyces*.

It has been reported by Robbins and Kavanagh (1937) that the pyrimidine and the thiazole components are utilized by *Phycomyces* in equi-molecular amounts. That this is qualitatively so has also been found during the course of the present experiments, and it has in addition been shown that the two portions are used in approximately equimolecular amounts

by the pea root (Bonner, 1938). This would be in agreement with the hypothesis that the two portions are used by the organism to synthesize thiamin itself. Experiments made in an attempt to actually demonstrate such a synthesis have thus far met with failure. Use has been made of the mold *Phytophthora cinnamomi* for which thiamin is an essential growth factor and upon which a pyrimidine-thiazole mixture is inactive (Robbins, 1938). Dried mycelium of *Phycomyces* which had been allowed to develop on the basic medium with pyrimidine-thiazole mixture as the

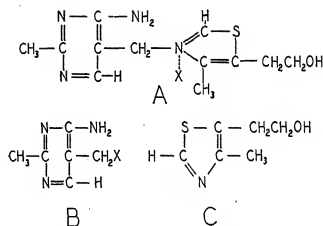


Fig. 1. A. Thiamin (vitamin B₁) molecule.—B. Pyrimidine portion of the molecule, ("vitamin pyrimidine").—C. Thiazole portion of the molecule ("vitamin thiazole"). The "X" on the methyl group in the 5 position of the pyrimidine ring may be Br, NH₂, etc.

growth factor was added in varying amounts to basic medium into which *Phytophthora* was inoculated. Although the *Phytophthora* flourished upon basic medium containing thiamin, it failed to develop either upon medium containing the *Phycomyces* mycelium or a pyrimidine-thiazole mixture. Schopfer (1938) has also been unable to demonstrate a synthesis of thiamin from the two constituents by *Phycomyces*.

ACTIVITIES OF ANALOGS OF THE VITAMIN THIAZOLE.—Analog² of the vitamin thiazole were tested in the presence of an equimolecular amount of the vitamin pyrimidine (A of fig. 1) for their ability to support the growth of *Phycomyces*. Activities will in all cases be expressed relative to the vitamin thiazole. Thus, an activity of 1 per cent indicates that the analog in question at a concentration of 10⁻⁶ molar gives the same dry weight of mold as does the vitamin thiazole at a concentration of 10⁻⁸ molar. All substances were tested at concentrations from 10⁻⁹ molar to 10⁻⁴ molar. The activities of twenty-two thiazoles are summarized in table 3. Number 1 of table 3 is the vitamin thia-

zole group is replaced by an NH₂ group. This is still less active than the Cl derivative, but here again it seems reasonable to suppose that hydrolysis to the vitamin thiazole may take place. In substance 4 the hydroxyl group is replaced by a fully methylated N atom. This, in contrast to the amino derivative, is completely inactive.³ An SH group replaces the hydroxyl group in substance 5. This substance again possesses a slight activity which may well be due to a small amount of in vivo hydrolysis. Replacement of the hydroxy-ethyl side chain with an unsubstituted ethyl group, a CH₂CN group, or an acetic acid group, as in substances 6, 7, and 8, results in a com-

TABLE 3. Activities of thiazole analogs in supporting the growth of *Phycomyces*.

Number	$ \begin{array}{c} \text{S} \quad \text{C}-\text{R}_3 \\ \diagup \quad \diagdown \\ \text{R}_2-\text{C} \quad \text{C}-\text{R}_4 \\ \diagdown \quad \diagup \\ \text{N} \end{array} $			Activity: %
	R ₂	R ₄	R ₃	
1 ^a	H	CH ₃	CH ₂ CH ₂ OH	100
2	"	"	CH ₂ CH ₂ Cl	10
3 ^b	"	"	CH ₂ CH ₂ NH ₂	0.6
4	"	"	CH ₂ CH ₂ N(CH ₃)Br	0.00
5 ^b	"	"	CH ₂ CH ₂ SH	0.8
6	"	"	CH ₂ CH ₃	0.00
7	"	"	CH ₂ CN	0.00
8	"	"	CH ₂ COOH	0.00
9 ^c	"	"	CHOHCH ₃	0.00
10 ^b	"	CH ₂ OH	CH ₂ CH ₃	0.00
11 ^d	"	CH ₂ CH ₂ Cl	CH ₃	0.00
12	CH ₃	CH ₃	CH ₂ CH ₂ OH	0.00
13 ^c	H	"	(CH ₃) ₃ OH	0.06
14 ^c	"	"	CH ₂ CHOHCH ₃	0.09
15	"	CH ₃	CH ₂ Br	0.00
16	"	"	CHO	0.00
17	"	"	COCH ₃	0.00
18 ^c	"	"	H	0.00
19	EtO	"	CH ₂ CH ₂ OH	0.00
20	NH ₂	"	CH ₂ CH ₂ OH	0.0
21	H	"	CH=CH ₂	0.1
22	methiodide of 1			0.00

^a This is the vitamin thiazole, B of figure 1.

^b Activity of picrate checks that of free base.

^c Activity of vitamin analog (see table 5) checks that of mixture of thiazole with pyrimidine.

^d Preliminary result.

zole and possesses an activity which is arbitrarily designated as 100 per cent. In substance number 2 the hydroxyl group of 1 is replaced by a Cl atom. This substance is one-tenth as active, under the conditions of our experiments, as the vitamin thiazole. It may, however, be hydrolyzed under suitable conditions in vitro to the vitamin thiazole, and it would seem reasonable to suppose that such hydrolysis may also take place in vivo. In substance 3 the hydroxyl

pletely inactive thiazole. In these three cases also, metabolism of the analog to the vitamin thiazole would be expected to be unlikely. With *Phycomyces* as with the pea root (Bonner, 1938) the hydroxyl group would seem to be of paramount importance for activity of a substance as a vitamin thiazole.

Substances 9, 10, and 11 represent three isomers of the vitamin thiazole (11 is an isomer of the halogen substituted active analog, 2), all of which are

² We are indebted to Dr. E. R. Buchman (California Institute) for supplying the synthetic thiazole analogs reported on here. This work was supported by a grant from the Research Corporation.

³ "Inactive" signifies that the substance in question must possess less than 0.01 per cent of the activity of the corresponding vitamin fragment when similarly tested.

inactive. Substances 12, 13, and 14 are three isomers containing one CH_2 group more than the vitamin thiazole. In 12, the extra group is in the 2 position which is normally unsubstituted, and the substance is inactive. Number 13 possesses a γ -hydroxy-propyl side chain rather than the β -hydroxy-ethyl side chain of the vitamin thiazole. This has a small but real activity, which, as is shown below, cannot be due to contamination by other active substances. In number 14 the hydroxyl group is present in the β position of the propyl side chain. This substance is still more active than number 13. Number 15 has one less CH_2 group than the vitamin thiazole. It is otherwise similar to number 2, but unlike it is completely inactive. From the substances 9 to 15 one can conclude that for activity as a *Phycomyces* growth factor there is a very considerable specificity as to the position of the thiazole hydroxyl group.

former investigators. Thus, the 5- β -hydroxy-propyl and 5- γ -hydroxy-propyl thiazole analogs have small but measurable activities, a conclusion compatible with the findings of Knight (1938) for *Staphylococcus*. (See also later section on activity of analogs of thiamin).

ACTIVITIES OF ANALOGS OF THE VITAMIN PYRIMIDINE.—Analog^a of the vitamin pyrimidine were tested in the presence of an equimolecular amount of the vitamin thiazole for growth activity upon *Phycomyces*. The activities of 11 pyrimidines are summarized in table 4. Number 1 is the vitamin pyrimidine itself and possesses a brommethyl group in the 5 position. Numbers 2 to 4 are similar to number 1 but have other substituents upon the 5-methyl group. An amino or an ethoxy group may replace the Br without diminishing the activity of the pyrimidine. A thio-formamidomethyl group on the other hand

TABLE 4. Activities of pyrimidine analogs in supporting the growth of *Phycomyces*.

$ \begin{array}{c} \text{N} \quad \text{C}-\text{R}_5 \\ \parallel \quad \parallel \\ \text{R}_2-\text{C} \quad \text{C}-\text{R}_3 \\ \parallel \quad \parallel \\ \text{N} \quad \text{C}-\text{R}_4 \end{array} $					
Number	R ₂	R ₄	R ₃	R ₅	Activity: %
1 ^a	CH ₃	H	CH ₂ Br	NH ₂	100
2	"	"	CH ₂ NH ₂	"	100
3	"	"	CH ₂ OEt	"	100
4	"	"	CH ₂ NHCHS	"	7
5 ^b	"	"	CN	"	0.03
6	"	"	CH ₂ COOH	"	0.00
7 ^c	"	"	CH ₂ CONH ₂	"	0.00
8	"	"	H	"	0.00
9	"	"	CH ₂ NH ₂	OH	0.00
10	"	"	CH ₂ OH	"	0.00
11	"	"	CH ₂ OEt	"	0.00

^a This is the vitamin pyrimidine, A of figure 1.

^b Preliminary result.

^c Eight other pyrimidines without reactive substituent upon the 5 methyl group found to be inactive but not listed.

In substances 12, 19, and 20 various groups are substituted upon the 2 position of the thiazole ring. These analogs possess little or no activity. Twenty-one contains a vinyl group in place of the hydroxyl-ethyl group of the vitamin thiazole and has only a very slight activity upon *Phycomyces*. The pea root (Bonner, 1938) apparently is able to metabolize this analog readily to an active thiazole, but the metabolic activities of *Phycomyces* seem to be in general more restricted in scope than those of the pea root. Number 22 is the methiodide of the vitamin thiazole—i.e., is a quaternary salt. The significance of its inactivity will be discussed more fully below.

These results are, in general, in agreement with those of Robbins and Kavanagh (1938) and of Schopfer (1937). However, wider ranges of concentrations have been used in the present work, and it has been possible to detect small activities which escaped the

seems, under the conditions used, to diminish the activity markedly. A CN in place of the substituted 5 methyl group appears to decrease the activity very greatly, although this result is of a preliminary nature. Less reactive substituents upon this 5 methyl group, such as a COOH group (number 6) or a CONH₂ group (number 7), result in complete inactivity. The substituents upon the 5 methyl group of substances 1 to 4 are all relatively reactive, and it is understandable that these pyrimidines could readily form quaternary salts with the thiazole, whereas in substances 6 and 7 this might not be expected. Substance 8, which has no substituent in the 5 position, cannot form a vitamin analog and is correspondingly inactive. For a substance to possess activ-

⁴ We are indebted to Dr. A. R. Todd (Lister Institute) and Dr. J. K. Cline (Merck and Co., Inc.) for supplying the synthetic pyrimidine analogs reported on here.

TABLE 5. Activities of thiamin (vitamin B₁) analogs in supporting the growth of *Phycomyces*.

Number	Vitamin Analog	Activity of: %	
		Vitamin Analog.	Corresponding pyrimidine-thiazole mixture
1 ^a ...	$ \begin{array}{c} \text{H} \\ \\ \text{N} - \text{C} - \text{NH}_2 \\ \quad \\ \text{CH}_2 - \text{C} = \text{C} - \text{CH}_2 - \text{N} \\ \quad \quad \\ \text{N} = \text{CH} \quad \quad \text{C} = \text{C} - \text{CH}_2\text{CH}_2\text{OH} \\ \\ \text{Cl} \end{array} $	100	100
2 ...	$ \begin{array}{c} \text{H} \\ \\ \text{N} - \text{C} - \text{NH}_2 \\ \quad \\ \text{CH}_2 - \text{C} = \text{C} - \text{CH}_2 - \text{N} \\ \quad \quad \\ \text{N} = \text{CH} \quad \quad \text{C} = \text{C} - \text{CH}_2\text{CHOHCH}_3 \\ \\ \text{Br} \end{array} $	0.1	0.09
3 ...	$ \begin{array}{c} \text{H} \\ \\ \text{N} - \text{C} - \text{NH}_2 \\ \quad \\ \text{CH}_2 - \text{C} = \text{C} - \text{CH}_2 - \text{N} \\ \quad \quad \\ \text{N} = \text{CH} \quad \quad \text{C} = \text{C} - (\text{CH}_2)_5\text{OH} \\ \\ \text{Br} \end{array} $	0.06	0.06
4 ...	$ \begin{array}{c} \text{H} \\ \\ \text{N} - \text{C} - \text{NH}_2 \\ \quad \\ \text{CH}_2 - \text{C} = \text{C} - \text{CH}_2 - \text{N} \\ \quad \quad \\ \text{N} = \text{CH} \quad \quad \text{C} = \text{C} - \text{CHOHCH}_3 \\ \\ \text{Br} \end{array} $	0.00	0.00
5 ...	$ \begin{array}{c} \text{H} \\ \\ \text{N} - \text{C} - \text{NH}_2 \\ \quad \\ \text{CH}_2 - \text{C} = \text{C} - \text{CH}_2 - \text{N} \\ \quad \quad \\ \text{N} = \text{CH} \quad \quad \text{C} = \text{C} - \text{H} \\ \\ \text{Br} \end{array} $	0.00	0.00

^a This is the vitamin itself.

ity as a vitamin pyrimidine, it is therefore necessary that it have a reactive, substituted 5-methyl group, permitting it to form a quaternary salt with the thiazole. A considerable number of further pyrimidines which were available for the present work did not possess such groups and were all inactive. They are not included in table 4, since they contribute nothing further to an understanding of the pyrimidine structural requirements.

The amino group in the 6 position of the vitamin pyrimidine cannot be replaced by an OH group, as is shown by the substances 9, 10, and 11. It seems probable that this amino group is to be regarded as

essential. The results on specificity of the pyrimidine half are in agreement with and extend those of Sinclair (1937) and of Robbins and Kavanagh (1938).

ACTIVITIES OF ANALOGS OF THE VITAMIN.—When a mixture containing analogs of one of the vitamin halves plus the appropriate other vitamin fragment is tested, at least two considerations might determine its activity: (1) ability of the organism to synthesize the component parts into an analog of the vitamin, if this occurs *in vivo*, and (2) the activity of the vitamin analog. The activities tabulated in tables 3 and 4 might be determined by either or both of these considerations. In order to determine whether syn-

min molecule, (2) that other organisms can utilize the two component parts, and (3) that these two parts are used in approximately equimolecular amounts would seem to suggest a resynthesis of the vitamin in the latter group and that this synthesis may be masked by secondary factors in the case of *Phycomyces*.

In investigations of the role of thiamin in the growth of pea roots use was made of the quantitative *Phycomyces* assay (Bonner, 1938). Because *Phycomyces* responds not only to thiamin but also to pyrimidine-thiazole mixtures, it is particularly useful for such studies, since a biological assay which determined only thiamin itself might fail to detect significant amounts of root growth factor activity.

As to the specificity of the pyrimidine structure required by *Phycomyces*, one can at present conclude that (1) a 6-amino group and (2) a reactive substituted 5-methyl group permitting the formation of quaternary salts with the thiazole are necessary. The same holds true for all organisms whose pyrimidine specificity requirements have been thus far investigated. Further conclusions cannot be drawn until more of the critical pyrimidines are available.

The thiazole requirements of *Phycomyces* are less strict than are those of the rat but are more so than are those of the pea root. Thus the vitamin analog of the 4-methyl, 5- β -hydroxy-propyl thiazole (number 2 of table 5) has an activity relative to the vitamin of 100 per cent upon the pea root, 0.1 per cent upon *Phycomyces*, and is inactive upon the rat (Buchman, 1938). The thiazole specificity of *Staphylococcus*, however, agrees well with that of *Phycomyces* (Knight, 1937, 1938). An hydroxyl group or a group readily metabolizable to such must be present in the thiazole molecule. Any change in position of this hydroxyl group from that which it occupies in the vitamin is attended by a great decrease or complete loss of activity on the growth of *Phycomyces*. The factors determining this specificity of position of the hydroxyl group are not as yet known. That an hydroxyl group is essential, however, is undoubtedly connected with the fact that with the vitamin, esterification with pyro-phosphoric acid yields co-carboxylase (Lohman and Schuster, 1937).

The factors influencing the differences between the thiazole requirements of different organisms are at present obscure. There are, however, at least two hypotheses which might be advanced to account for this behavior: (1) The carboxylase proteins of these different organisms may differ in their thiamin specificity requirements for the formation of an active enzyme; (2) vitamin B₁ may possess more than one physiological function. Different physiological functions might well demand different structural requirements. In any one organism the physiological function of greatest importance in that organism would deter-

mine the structural specificity. Vitamin B₁ itself would then be an integrated structure possessing the optimum configuration for all of these activities.

Which of these or other possibilities is the correct answer to this problem must await the outcome of further experiments.

SUMMARY

The chemical specificity of thiamin (vitamin B₁) as a growth factor for *Phycomyces Blakesleanus* has been investigated. The work of others indicating that *Phycomyces* is able to utilize as a growth factor mixtures of the pyrimidine and thiazole components of the vitamin as well as the vitamin *per se* has been confirmed, and it has been shown that such mixtures have quantitatively the same activity as the vitamin itself.

The fact that *Phycomyces* reacts to a mixture of vitamin pyrimidine and vitamin thiazole does not invalidate its use in biological assays for "vitamin B₁ activity" in investigations concerning the root growth of higher plants. The plants which have been thus far investigated are also able to utilize such mixtures.

It has been impossible to demonstrate an *in vivo* synthesis by *Phycomyces* of thiamin from pyrimidine and thiazole. It is deemed possible that such a synthesis may occur, but that it is masked by unrecognized secondary factors.

A substituted thiazole to be active as a vitamin thiazole must possess (1) a free nitrogen atom which may form a quaternary salt with the vitamin pyrimidine, and (2) an hydroxyl group or a group readily metabolizable to such. If this hydroxyl group is removed to a position other than that which it occupies in the vitamin thiazole, the activity of the analog is either greatly diminished or completely abolished. Several thiazole analogs are shown to have definite, although small, activities. Substitution upon the 2 position of the thiazole nucleus results in an analog inactive in supporting the growth of *Phycomyces*.

A substituted pyrimidine, to have activity as a vitamin pyrimidine, must possess (1) a reactive substituted 5-methyl group capable of yielding quaternary salts with the thiazole nucleus and (2) a 6-amino group (the amino group of the vitamin pyrimidine cannot be replaced by an hydroxyl group).

Vitamin analogs consisting of vitamin pyrimidine combined with thiazole analogs possess activities substantially identical to those obtained when the same thiazole analog is supplied by *Phycomyces* in a mixture with the vitamin pyrimidine. *In vivo* synthesis to the vitamin analog, if it occurs, does not limit the activity of these substances.

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CORONASTRUM: A NEW GENUS OF ALGAE IN THE FAMILY SCENEDESMACEAE¹

R. H. Thompson

IN JULY, 1937, a new species of algae was collected from the very polluted water of a drainage ditch near Lawrence, Kansas. It is so strikingly different in morphology from all other described species of green algae that it is here described as the type species of a new genus in the family Scenedesmeaceae.

Coronastrum gen. nov.—Cellulis pyriformibus aut globosis, cum uno chromatophoro parietale et uno somate pyrenoideo in cellula quaque; cellulis quattuor in coenobio quoque, in lamina plana (quae postea distorta est) collocatis, in angulis quadrati positae, inter se remotis, sed per fibras e materia muri cellulae formatas conjunctis; cellula quaque fragmento muri cellulae maternae, squamae simili, praedita. Coenobii quattuor usque ad sedecim per fibras e materia muri cellulae formatas inter se saepe conjunctis; coenobio quoque coloniae ad angulum quadrati adjuncto sed plano coenobii cuiusque planum coloniae omnis cum rectis angulis secante.

¹ Received for publication August 11, 1938.

I wish to express my gratitude to Dr. A. J. Mix for the help and criticism he has given throughout this study and to Miss Winnie D. Lowrance for the editing of the Latin diagnoses. I am also much indebted to Dr. Gilbert M. Smith of Stanford University for his advice on the taxonomic relationships of this alga.

Cells pyriform or globose, each containing one parietal chloroplast and one pyrenoid; coenobia composed of four cells arranged in a flat plate (which later is distorted) at the angles of a square, remote from one another but connected by strands of cell-wall substance; each cell bearing a scale-like fragment of mother-cell wall. Four to 16 coenobia may be united by strands of cell-wall substance; each coenobium attached at the corners of a square, but with the plane of each coenobium at right angles to the plane of the colony as a whole.

The name *Coronastrum* was chosen to describe the characteristic colonial form which suggests a crown.

Coronastrum aestivale sp. nov.—Cellulis pyriformibus aut globosis, 3.3-6.6 μ diam., cum uno chromatophoro parietale et uno somate pyrenoideo in cellula quaque; cellulis quattuor in coenobio quoque, in lamina plana (quae postea distorta est) collocatis, in angulis quadrati positae, inter se remotis, sed per fibras e materia muri cellulae formatas conjunctis; cellula quaque fragmento muri cellulae maternae, squamae simili praedita. Coenobii quattuor usque ad sedecim per fibras e materia muri cellulae formatas inter se saepe conjunctis; coenobio quoque coloniae ad angulum quadrati adjuncto sed plano coenobii

eiusque planum coloniae omnis cum rectis angulis secante.

Cells pyriform or globose, 3.3–6.6 μ in diameter, each containing one parietal chloroplast and one pyrenoid; coenobia composed of four cells arranged in a flat plate (which later is distorted) at the angles of a square, remote from one another but connected by strands of cell-wall substance; each cell bearing a scale-like fragment of mother-cell wall. Four to 16 coenobia may be united by strands of cell-wall substance; each coenobium attached at the corners of a square, but with the plane of each coenobium at right angles to the plane of the colony as a whole.

Collected in a ditch near Haskell Institute, Lawrence, Kansas, July 6, 1937, July 5, 1938. R. H. Thompson. Type material preserved in formalin-acetic-alcohol fixative and some mounted on slides deposited in the herbarium of the University of Kansas.

As has been stated in the description, mature colonies may be formed by four or more coenobia remaining attached after reproduction (the greatest number observed in culture was 16, making a colony of 64 cells). The sixteen-celled colony is more commonly found. In these the coenobia are arranged at the corners of a square and connected by clear, thick strands of cell-wall substance; but they are so arranged that the plane of each coenobium lies at right angles to the plane of the colony as a whole. The colony or compound coenobium is then twisted as though one had taken opposite sides in his hands and had warped them in opposite directions about ten degrees.

Juvenile cells measure 3.3 μ in diameter, adult cells 6.6 μ in diameter. In shape they may be pyriform, broadly top-shaped or globose. The chloroplast is single and parietal, lying in the outer, larger part of the cell and containing one pyrenoid. The cells of a coenobium as well as the coenobia are 3.3–5 μ apart but connected by strands of cell-wall substance. Each cell is subtended by or bears on its inner margin a scale-like fragment of mother-cell wall which is broad at the point of attachment to the cell and which tapers to a point and arches over the cell slightly.

In the coenobia the cells are oppositely arranged in one plane to form a square. The larger rounded ends are directed outward, and the smaller, hyaline ends are directed inward and joined to one another by strands. The coenobia are joined to form a colony by strands from two diagonally opposite cells of a coenobium to the corresponding cell of the coenobium adjacent on each side (fig. 2). How this comes about may be understood from a study of its asexual mode of reproduction.

Fragmentation of the colony was observed in culture with each ultimate fragment consisting of a single coenobium, the cells of which were of adult size. Reproduction is by means of autospores, four autospores being produced by each cell. The cell wall swells, appearing to become of a watery consistency within and so thin on the outside as to be almost indiscernible (fig. 4, 5, 6, 7). The protoplast in the

meantime has undergone two successive divisions that are longitudinal—i.e., extending from the inner to the outer pole of the mother cell, resulting in a four-lobed structure (fig. 6). The plane of one division is the same as the plane of the mother coenobium, and the plane of the other division is at right angles to the plane of the coenobium. Here can be seen the origin of the twisted appearance of the fully developed sixteen-celled colony, since, of the two daughter cells that eventually remain attached to the framework of the mother coenobium, one lies just above and the other just below the plane of the mother coenobium. Their connections to the cells adjacent on each side must necessarily be at a slight angle. As the cells become more widely separated, these oblique tensions at each end distort the flat plate arrangement. The four lobes or cells separate from each other at the

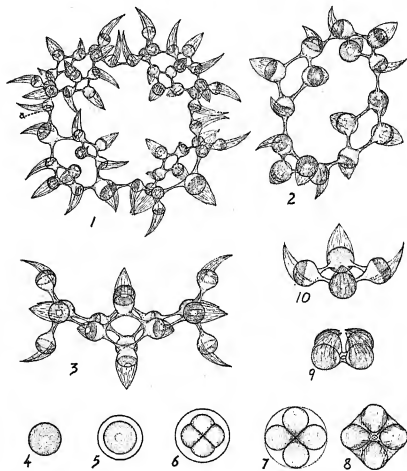


Fig. 1–10. *Coronastrum aestivale* sp. nov.—Fig. 1, 2, 3. Compound coenobia. $\times 625$.—Fig. 4, 5, 6, 7, 8, 9, 10. Stages in reproduction. (Semidiagrammatic.)

outer ends first, the inner ends remaining in proximity for a time (fig. 7). During the separation of the daughter cells, a split appears in the old mother-cell wall at the inner end between the two opposite cells that eventually remain attached to the mother coenobium framework and widens considerably at right angles (fig. 8). This splitting separates the four daughter cells and produces the strands that keep them united into one coenobium. Continued separation of the cells by addition of cell-wall substance to the connecting strands and continued increase in size of the individual cells results in the rupturing of the outer portion of the old mother-cell wall, which in the meantime has become very thin (fig. 8). The

ruptures occur between the individual daughter cells, thus leaving a scale-like fragment attached at the inner end of each (fig. 9, 10). The daughter cells are $3.3\ \mu$ in diameter at this point and increase in size to $6.6\ \mu$.

In the above described manner, each cell of a coenobium gives rise to a new coenobium, though it may not do so at the same time as the others. Various stages were observed in culture: ones in which there were three adult cells and one juvenile coenobium; ones in which there were two adult cells and two juvenile coenobia; ones in which there were three juvenile coenobia and one adult cell; and ones in which there were four juvenile coenobia completing the sixteen-celled colony. Further reproduction of each cell in the sixteen-celled colony ultimately results in a sixty-four-celled colony or multiple coenobium composed of 16 coenobia (fig. 1). Multiple coenobia of 64 cells were seen in culture in which the individual cells were again reproducing, though no multiple coenobia were seen in which the 64 cells had all fully divided to form a colony of 256 cells. This might well happen if the plant were not disturbed by water currents. However, under the cultural conditions in which this alga was found, the sixty-four-celled coenobia usually underwent fragmentation; and, in such cultures, the type of multiple coenobium most commonly found was the sixteen-celled one. In changing from the sixteen- to the sixty-four-celled condition, those cells which were concerned in the connecting of the coenobia to form the sixteen-celled colony divide in the same manner as the rest; but, of the two daughter cells that would usually remain free in the formation of a sixteen-celled colony, the lowermost or the one towards the center of the colony remains attached to the old colonial strand (fig. 1a).

Coronastrum aestivale is being placed in the family Scenedesmaceae because its reproduction is asexual, and the asexual spores remain attached to form an autocolony in which the cells have a definite orientation. It is being located in the neighborhood of the genera *Crucigenia*, *Hofmania*, and *Tetrastrum* because each single coenobium contains four cells which are quadrately disposed in a flat plate. It is similar to *Hofmania* in that each cell of a coenobium bears a scale-like fragment of mother-cell wall, but it differs from members of all three genera in that the cells of a coenobium are remote from one another and connected by definite strands of mother-cell wall substance. It differs from members of the genus *Tetrastrum* in that the cells do not bear spines as do those of *Tetrastrum*. It also differs from members of the genera *Crucigenia* and *Hofmania* in the formation of

compound coenobia. In the compound coenobia of *Crucigenia* and *Hofmania*, the individual coenobia are arranged in a flat plate and are connected by old mother cell-wall fragments, or the whole may be enclosed in a gelatinous envelope. In *Coronastrum aestivale* the compound coenobia have no gelatinous envelope, the coenobia are not arranged in a flat plate but rather in a hoop-like manner with the planes of the individual coenobia and the colony opposed, and the coenobia are connected by strands of mother-cell-wall substance of a definite shape and texture rather than by fragments.

In the collections taken during the periods of observation each of the two summers, the following associated algae were found: *Chlamydomonas* sp., *Oocystis gigas* Archer var. *Borgei* Lemm., *Pandorina morum* Bory, *Errerella bornheimiensis* Conrad, *Euglena proxima* Dang., *Coronastrum aestivale*, *Dimorphococcus lunatus* A. Braun, *Coelastrum sphaericum* Näg., *Scenedesmus obliquus* (Turp.) Kütz., *Actinastrum Hantzschii* Lagerh., *Gonium pectorale* Muell., *Schizomeris leibleini* Kütz. These organisms formed a crumbly scum on the surface of the water. The water was the most putrid from which the author has ever collected. It was highly polluted with fecal material and supported an abundance of protozoan forms, particularly *Vorticella* spp. and *Charachium* spp., which clothed every submerged stick with white, feathery masses. The algae bloom came up both summers after a week of hot weather without rain. In 1937, the temperature of the air was 104°F . the day of the collection. In 1938, it was 100°F .

SUMMARY

A new genus, *Coronastrum*, is described in the family Scenedesmaceae near the genera *Crucigenia* and *Hofmania*. The cells are pyriform to globose, each containing a single parietal chloroplast with one pyrenoid. Four cells are connected by strands of cell-wall substance to form a coenobium. Compound coenobia may contain 4, 16, or 64 coenobia.

Coronastrum aestivale is described as the type species. Cells $3.3\text{--}6.6\ \mu$ in diameter. There is no gelatinous, colonial envelope. The alga is planctonic in polluted waters.

C. aestivale is placed in the family Scenedesmaceae because its reproduction is asexual and the asexual spores remain attached to form an autocolony in which the cells have a definite orientation.

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VEGETATIVE REPRODUCTION IN CHLOROPHYTUM ELATUM¹

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THE PLANT commonly known as *Chlorophytum elatum* R. Br. is easily propagated by small plants which develop at the tips and nodal regions of stolons. Scores of such individuals may be found on one mature plant, and no doubt such ease of propagation has helped to make this plant a favorite greenhouse subject. Other members of the Liliaceae, especially the closely related genus *Anthericum*, commonly known as St. Bernard's lily, illustrate this same method of rapid vegetative reproduction. This study was made to determine the origin of these new plants and of the stolons upon which they are produced.

Bailey (1925) states that the scapes of *C. elatum* are sometimes transformed into stolons which bear leafy offshoots. Fairburn (1936) in a general study on plant propagation mentions that *Anthericum* is reproduced by offsets attached to the parent by a short stem. Gravis and Doneel (1900) in speaking of the flower-stalk of *C. elatum* say that sometimes an axis corresponding to the peduncle is transformed into a foliated shoot capable of taking root. Laurie and Chadwick (1931) list *Anthericum Liliago* as reproducing by offsets and stolons.

Most of the investigations have been concerned with practical methods of propagation and little with the histology of the origin of new plants. Detailed studies would give a more nearly complete understanding of cellular changes as related to the formation of new structures in vegetative reproduction.

EXPERIMENTAL PROCEDURE.—The plants used for the investigation were grown from a single original plant obtained through the courtesy of the Missouri Botanical Garden. Thirty young plants, from four to six inches high, were removed from the stolons in late September and placed in ordinary potting soil in the greenhouse. Later plantings were made at about four-week intervals. Early growth was rather slow, but after two months all the plants showed very vigorous growth and many new stolons.

Material was removed for study at various intervals as soon as the young stolons first made their appearance. Such sections included the embryonic stolon, tips and nodes of the young stolon, and different stages of development of the young plants on the stolon. Material was fixed in the usual formalin-acetic-alcohol mixture, dehydrated with alcohol, and cleared in xylene. Paraffin sections were cut 8-12 microns in thickness and stained in Heidenhain's iron-alum haematoxylin. Free hand sections were also generally employed throughout the investigation.

GENERAL MORPHOLOGY.—*Chlorophytum* is herbaceous, with a much shortened stem, long linear leaves, and rather fleshy roots. The flower scape is long and slender and considerably branched. It is almost erect when young but soon becomes procumbent, and from it the flowers and fruits as well as the new plants are

developed (fig. 1). The vegetative structure which serves to reproduce the plant may be called a scape. However, according to more general usage, the term stolon applies and is used in this discussion.

VEGETATIVE REPRODUCTION.—The stolon is morphologically an elongation of the very short erect stem of the parent plant. It consists of a primary axis and several branches termed secondary axes. At the tip of each stolon there is produced a rosette of new plants. The number varies from one to as many as ten. The average count for fifty primary axes was six. Among these terminally borne plants may also be found a number of flowers and sometimes fruits (fig. 2).

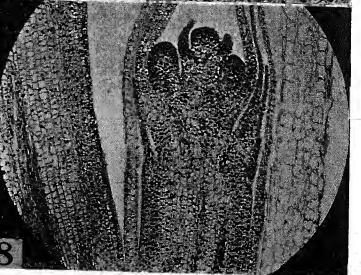
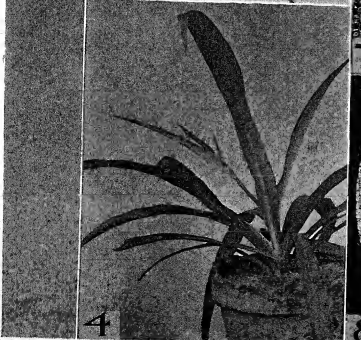
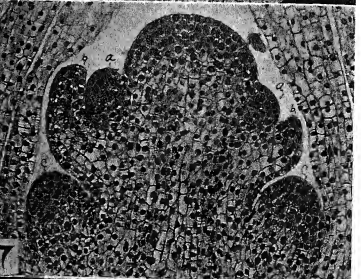
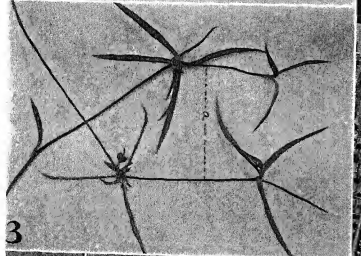
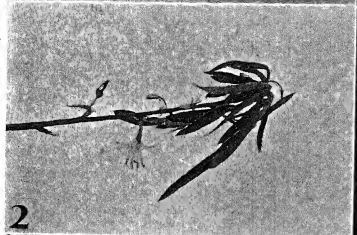
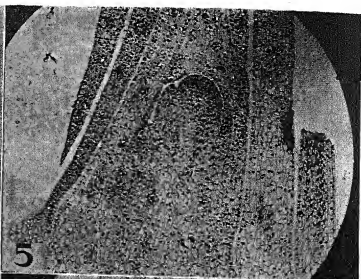
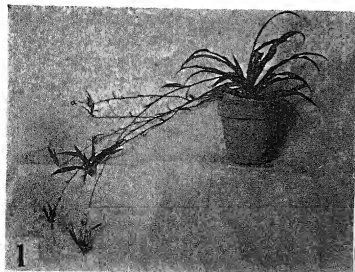
The upper portion of the primary axis has some three to ten nodes, from each of which develops a small bract, several flowers, and one or two new plants. The development of flowers and new plants at these nodes does not follow any fixed pattern. Occasionally only flowers may develop. The number of flowers appearing at these nodes varies from one to five (fig. 13, 14). In some instances the production of plants may not take place until some time after the flowers have appeared.

From each of the lower nodes of the primary axis develop three to five secondary axes. The average number appears to be about three. The external appearance of the secondary axis is very similar to the primary one. At the apex of each there is also developed a cluster of new plants. Sometimes, particularly if the apex of a secondary axis is injured, a tertiary axis may be produced which duplicates the structure of the other axes. Occasionally after the growth of the primary or secondary axis has apparently terminated, another axis may form and produce at its apex a single new plant (fig. 3). On some primary axes the lowermost bract subtends a new plant only, and flowers are never produced (fig. 11). Sometimes a new plant is formed in the axil of a primary axis or of a secondary axis (fig. 12).

Thus it is found that new plants develop vegetatively (1) at the apices of primary, secondary, and tertiary axes; (2) at the nodes of the primary and secondary axes; and (3) in the axils of such axes.

The time and order of development of the new plants is not constant. Frequently the rosette of plants at the apex of the primary axis develops first, usually followed by those at the apices of secondary axes. The next to develop are usually those at the anterior nodes of the primary axes, followed in turn by those at the nodes of the secondary axes. The new plants develop semi-parasitically upon the parent for some time, regardless of their position. Several foliage leaves are produced, and short aerial roots usually develop from the cluster of plants at the tip of the stolon (fig. 9).

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ORIGIN AND DEVELOPMENT.—The stolon may originate either from near the apical meristem of the stem proper or from axillary buds. A considerable amount of leaf and stem tissue develops as the stolon matures, with the result that the basal part becomes deeply embedded in stem tissue (fig. 15). It was difficult to obtain sections to show very early stages, but the first stolon to appear develops immediately after the initial cells have been cut off by the growing point of the main stem tip. Successive stolons are developed from buds in the axils of foliage leaves (fig. 10). A longitudinal section through a bud discloses a convex cushion of meristematic cells enclosed in an imbricated manner by leaf and bract primordia. Although the morphological appearance of these axillary buds is the same, it is impossible to predict the course of their development. Some may grow into secondary axes and, according to Gravis and Doneel (1900), may develop into flower-stalks.

The first macroscopic indication of the stolon is an elongated bract-enclosed structure growing out from near the center of the plant (fig. 4, 16). Microscopically it may be recognized by the nature of the derivatives of the apical meristem (fig. 5). The growing point of the stolon is a mass of meristematic cells which develop into the lateral protuberances. The first of these becomes the elongated basal bract. In very early development there may be seen in the axil of this bract a mass of meristematic cells (fig. 6). Identical structures are cut off at each of the succeeding two or three nodes, and from the lower nodes of the primary axis these cells become the growing points of secondary axes. The rate of elongation of the bract exceeds that of the growing point so that the bract becomes mature early in the ontogeny of the axis and forms a protective envelope covering the apical meristem.

In the axils of the bracts flowers, or flowers and additional bracts, develop. After the initials of the secondary axis are laid down, the first lateral protuberance becomes a bract. At the base of the bract another mass of meristematic cells becomes the growing point of a very short axis (fig. 7). The first derivative of this apical region forms later the flower bud. At the base of the pedicel of the flower a mass of initiating cells remains which may develop either into additional flower buds or into the first leaf primordium of a new plant. These early derivatives do not follow any regular order of development. Frequently all the flowers may bloom before there is any evidence of the leaves of a new plant, or the leaves may appear at the same time as the third or fourth flower. Since most of the early structures of the axis are differentiated when the stolon is but a few millimeters in length these leaf primordia are covered by mature bracts arising at the nodes below.

In the axils of the new leaf primordia are found typical convex growing points which usually function as the apical meristem of the new plant. Some of the derivatives of these pro-meristems may be flower primordia. However, in the formation of the terminal plants the leaves nearly always develop first, or together with the flowers. The development of the secondary axis is almost identical with that of the primary one (fig. 8). Briefly, the apical meristem of the stolon, which is itself a segment of the apical meristem of the stem tip of the parent plant, becomes subdivided to form the bracts and flowers found on the stolon and also the new plants.

Roots.—Aerial roots, resembling those produced by some orchids, are formed at the base of the plants in the terminal rosette (fig. 9). These could not be induced to grow into normal roots even when placed in moist sand. The normal feeding roots grow out from the base of each plant soon after placing such an individual in moist soil or sand. They develop rapidly and soon become more or less fleshy. These roots arise adventitiously from thin-walled parenchymatous cells lying near the vascular bundles at the base of the plant close to where it was originally attached to the stolon.

Discussion.—As regards the types of cells involved in vegetative reproduction, plants may be grouped into two general classes. In the first the growth of new parts is made possible by the activity of meristematic cells which can be traced directly to other such cells of the plant body. This is illustrated by the formation of roots from the cambium at the base of a cutting or the development of a shoot in a leaf axil from embryonic cells which were laid down earlier by the stem tip primordium. In the second instance the newly formed parts originate in morphologically differentiated cells ordinarily not considered embryonic, such as those of the epidermis, parenchyma of root and stem, phloem, and sometimes the xylem.

The histological findings presented here show that the plants formed on the stolons of *C. elatum* are produced by the continued activity of meristematic cells. Mann (1930) reported that new plants in the strawberry, *Fragaria virginiana* var. *chiloensis*, are developed immediately from the growing point of the stolon and that the new roots arise adventitiously from the stem. This development is somewhat similar to that found in *C. elatum*. As regards the origin of the new parts this type of vegetative reproduction is very different from that already reported in *Saintpaulia ionantha* Wendl. by Naylor and Johnson (1937) and in *Crassula multicava* by McVeigh (1938). In both of these latter species the new plants arise from morphologically differentiated cells, particularly epidermal cells.

Fig. 1-8.—Fig. 1. Whole plant showing procumbent nature of the stolons and the production of flowers and new plants.—Fig. 2. Tip of the stolon with rosette of new plants, also the flowers and fruits.—Fig. 3. Showing development of a short axis and single new plant which occurs occasionally from the terminal cluster.—Fig. 4. Plant about four weeks old with first stolon.—Fig. 5. Earliest indication of the stolon from the stem tip.—Fig. 6. Section through the growing point of the stolon.—Fig. 7. Apical meristem of the stolon. (a) and (c), primordia of secondary axes; (b), bracts.—Fig. 8. Showing formation of secondary axis. Portion of primary axis to the left.

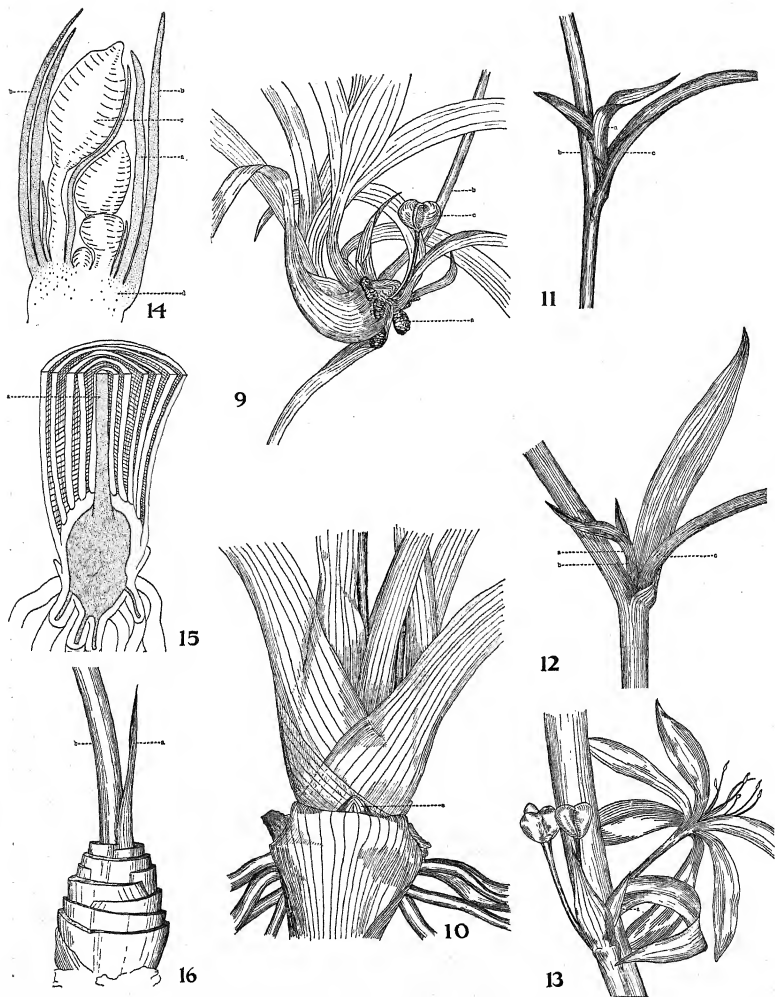


Fig. 9-16.—Fig. 9. Terminal rosette of plants and the stolon (b), fruit (c), and aerial roots (a).—Fig. 10. Stolon arising as an axillary bud.—Fig. 11. Production of new plant (a) in the axil of the bract (c).—Fig. 12. Showing production of new plant (a) in the axil of the primary and secondary axis.—Fig. 13. Formation of flowers, fruits, and the first leaves of a new plant (a) at the nodal region.—Fig. 14. Section through a bud of the stolon showing leaf primordia (a), bracts (b), flowers (c), and stem primordium (d).—Fig. 15. Diagram to show the relation of the stolon (a) to the sheathing leaf bases.—Fig. 16. Basal part of the parent plant with the laminae of the leaves removed showing stolon (b) and an elongated axillary bud (a).

The development of the stolon and new plants in *C. elatum* does not differ from the usual process of axis elongation and branching found among other members of the monocotyledons. Stout (1937) has described the proliferations which appear on the flower scapes of certain of the daylilies, *Hemerocallis*. These structures arise as buds in the axils of bracts at the nodes and develop into small plants. After the blooming period such plants are removed and treated as cuttings. Traub (1937) has also reported aerial plants on the flower scapes of the same plant. He mentions induced formation by planting portions of the scape treated or not treated with growth substances. *Anthericum liliago*, usually confused with *Chlorophytum*, also produces scores of new plants on the flower scape. Histological investigations have not been made in any of these plants as far as the writers know. It is not definitely known to what extent the *Anthericums* produce such proliferations in their native habitat. Examination of a number of specimens in the herbarium of the New York Botanical Garden did not disclose any such structures.

SUMMARY

Chlorophytum elatum produces new plants vegetatively at the nodes and the apex of the stolon and in the axils of the leaves.

The new plants (with the exception of the roots) originate from meristematic cells.

Roots are initiated adventitiously from thin-walled parenchymatous cells near the vascular system.

There is no evidence that the shoots of the new plants originate from morphologically differentiated cells.

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INFLUENCE OF DEUTERIUM OXIDE ON THE GROWTH OF CHLORELLA VULGARIS¹

Robertson Pratt

EXPERIMENTS HAVE shown that the rates of photosynthesis and respiration in the unicellular green alga *Chlorella vulgaris* are about 40 per cent and 60 per cent, respectively, as great in buffers prepared with 99 per cent deuterium oxide, or heavy water, as in those prepared with ordinary water (Curry and Trelease, 1935; Craig and Trelease, 1937; Pratt and Trelease, 1938).

Since these processes furnish organic materials and energy required for growth, a study was made of the influence of D₂O on the growth of *Chlorella*. Reitz and Bonhoeffer (1935) showed that the unicellular green alga *Scenedesmus* sp. did not grow in 98.4 per cent D₂O, and Delamater (1936) observed that concentrations of D₂O of 48.1 per cent or above reduced the rate of fission and other physiological activities in the protozoan *Uroleptis mobilis*; almost immediate death occurred in 98 per cent D₂O. Meyer (1936), however, reported that *Chlorella vulgaris* grew as well in a nutrient solution prepared with 99.2 per cent D₂O as in one prepared with H₂O.

MATERIALS AND METHODS.—The D₂O was prepared by the method described by Brown and Daggett

(1935) and was then purified by four successive distillations, as in previous experiments. The ordinary control water (H₂O containing 0.02 per cent D₂O), obtained from a laboratory still, was redistilled in the same manner as the D₂O. The different D₂O-H₂O mixtures used were prepared by diluting D₂O with the control water.

Cells for the experiment were taken from a four-day-old, rapidly growing culture of *Chlorella vulgaris* grown as previously described (Craig and Trelease, 1937) in a solution that contained KNO₃, 0.025 M; MgSO₄·7H₂O, 0.02 M; KH₂PO₄, 0.018 M; FeSO₄·7H₂O, 0.00001 M (with an equal concentration of potassium citrate), and a stock solution of micro-metabolic elements (Trelease and Trelease, 1935). The pH of this solution was 4.5. Cells were separated from the culture medium by centrifugation and decantation and were rinsed twice with distilled water in the same way. They were then suspended in a small volume of distilled water. The density of population in the suspension was estimated from haemocytometer counts, and inocula containing approximately 100,000 cells (0.5 ml.) were transferred to 50 ml. Pyrex glass Erlenmeyer flasks, each of

¹ Received for publication September 1, 1938.

which contained 10 ml. of the nutrient solution prepared with D_2O , H_2O , or different D_2O - H_2O mixtures. Initially there were approximately 10 cells per c.m.m. of solution in each flask.

The flasks were suspended in a water bath (temperature, 18° - $20^\circ C.$) and were illuminated continuously by a battery of twenty-four 50-watt frosted Mazda lamps 23 cm. below them. The light intensity, measured by means of a Weston photoelectric-cell light meter, Model 603, varied from 23,000 lux at the beginning of the experiment to 18,000 lux at the end. A dry gas mixture containing 5 per cent CO_2 and 95 per cent air was bubbled continuously into the flasks through separate inlet tubes. The cultures were vigorously shaken daily to insure uniform suspension of the cells.

Growth measurements were made periodically by withdrawing a small sample of the suspension from each flask and estimating the cell population by averaging three haemocytometer counts. The work was done in the laboratory of plant physiology at Columbia University.

RESULTS.—The growth of *Chlorella* in the nutrient solutions prepared with the different D_2O - H_2O mixtures is shown in figure 1. It is apparent that the growth of the alga was markedly decreased as the proportion of D_2O in the solution was increased. Very little growth occurred in 75 per cent D_2O . Final populations and maximum rates of multiplication are

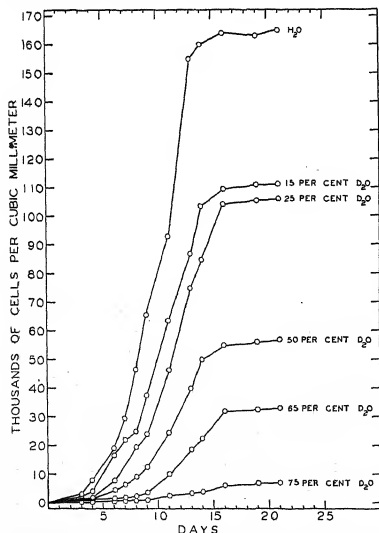


Fig. 1. Growth of *Chlorella vulgaris* in nutrient solutions containing different concentrations of heavy water.

shown numerically in table 1. The maximum rates of increase in cell number were estimated by computing the slopes of the steepest portions of smoothed curves drawn through the points plotted in figure 1. Figure 2 shows that these rates were approximately inversely proportional to the percentage of D_2O initially present in the solution. No difference was detected in the average size of the cells in the different solutions.

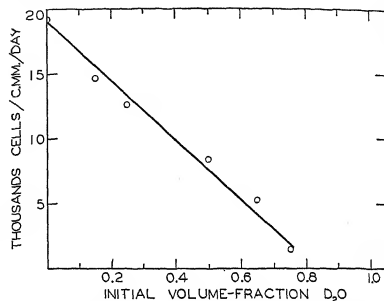


Fig. 2. Maximum rate of increase in cell number as a function of the fraction of heavy water initially present in the nutrient solution.

Growth of *Chlorella* over a period of many days was depressed by D_2O much more than photosynthesis and respiration were depressed during relatively brief periods.

The results of these experiments are similar to those of earlier studies on the growth of *Erysiphe* germ tubes (Pratt 1936, 1937) and the roots of higher plants (Pratt and Curry, 1937). Increase in the size of the germ tubes was due primarily to cell enlargement (probably accompanied by nuclear multiplication); cell multiplication had little, if any, part in their growth. Likewise, measureable growth of the

TABLE 1. Growth of *Chlorella vulgaris* in nutrient solutions prepared with different D_2O - H_2O mixtures.

Initial fraction of D_2O by volume	Final number of cells (thousands per c.m.m.)	Maximum rate increase (thousands per c.m.m. per day) ^a
0.00	165.0	19.3
0.15	111.5	14.7
0.25	106.0	12.1
0.35	82.5	8.7
0.50	57.0	5.2
0.65	32.8	1.3
0.75	7.3	

^a Slope of steepest portion of smoothed curves drawn through points shown in figure 1.

roots that were studied was due principally to cell enlargement. But in the present investigation a period of cell enlargement preceded each division, and growth depended upon both cell multiplication and increase in cell size. It is not yet possible to tell whether the several interdependent processes involved in growth are equally susceptible to the harmful action of D_2O or whether they respond independently to its action. It should be observed, however, that the general relationships between maximum rate of growth and fraction of D_2O initially present are somewhat similar for both *Erysiphe* and *Chlorella*. Likewise, the proportion of heavy water in the D_2O - H_2O mixture that checked growth was about the same for both organisms. This may mean that D_2O influences the growth of *Chlorella* chiefly through its action on cell enlargement.

SUMMARY

A study was made of the growth of the unicellular green alga *Chlorella vulgaris* in nutrient solutions prepared with different concentrations of deuterium oxide, or heavy water. Light of high intensity and a gas mixture that contained 5 per cent CO_2 and 95 per cent air were supplied continuously. In contrast to the report of Meyer (1936), it was found that the amount of growth of the alga decreased as the proportion of D_2O initially present in the solutions increased. Practically no growth occurred in 75 per cent D_2O . The maximum rate of growth was an inverse approximately linear function of the initial fraction of D_2O in the solution.

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THE EFFECT OF LIGHT ON THE ACCUMULATION OF ASCORBIC ACID IN YOUNG COWPEA PLANTS¹

Mary Elizabeth Reid

IT HAS been well established that dry seeds contain no ascorbic acid. However, if exposed to conditions suitable for germination, they will begin to accumulate it within a fairly short time. The period required for the appearance of the vitamin in measurable quantities varies in different types of seedlings, a longer time being essential in cereals (Fürst, 1912; Kucera, 1928; Harden and Zilva, 1924) than in legumes (Simonik, 1929). Most of the earlier studies have been concerned with the antiscorbutic potency of the entire sprouted seed and have been conducted chiefly by investigators in the field of animal nutrition. Recently, more detailed studies have been reported in which the plant material used for the

quantitative tests was both selected and investigated with greater discrimination and more attention given to factors causing variations than was characteristic of most of the earlier work.

In many investigations the quantitative distribution in different organs of the plant has been determined (Bessey and King, 1933; Clark, 1937; Bogart and Hughes, 1935; Levy and Fox, 1935; Rudra, 1936; Hou, 1937; Glick, 1937; Reid, 1937), and in others attempts have been made to localize the distribution within the tissues composing the organs (Strohecker, 1935; Bourne and Allen, 1935; Glick, 1937; Reid, 1937). By observing the effects of silver nitrate dissolved in dilute acetic acid on tissue sections, the localization of reduction effects within the cell itself has been investigated (Geitler, 1922; Gautheret, 1935; Weber, 1937; Dischendorfer, 1937). The value of these tests depend upon the assumption that ascorbic acid is the only substance known in living tissues

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which has the capacity to reduce readily the acidified silver nitrate solution. These studies are held to show that the chloroplasts are the chief centers of reducing action in the cells. Mirimanoff (1938), however, has reported that he found the reducing action to occur within the vacuoles and not within or at the surface of the plastids. He contends that the reduction effects previously mentioned were largely due to photolysis. Since a number of substances which may be contained in living cells will reduce silver nitrate under the influence of light, it is important that care should be taken to protect the tissues subjected to the tests from exposure to light. This precaution was not followed in much of the work which has been done on the subject. A satisfactory settlement of the problem has not yet been reached.²

The accumulation of ascorbic acid in seedlings at different stages of growth has also been studied (Matsuoka, 1935; Strohecker, 1935; Bogart and Hughes, 1935; Lee, 1936; Rubin and Strachizky, 1936; Glick, 1937; Reid, 1937). In seedlings grown in darkness this accumulation continues for several days, the exact time depending on the kind of seedling and the external conditions for germination such as temperature, aeration, and moisture. The present investigations show that shortly after the supply of usable organic food reserves has been exhausted, there is a period in which no further accumulation occurs, and this is followed by a decline in the quantity of ascorbic acid during the remainder of the life of the seedling. An increase in ascorbic acid has been observed to occur during growth, at least up to its later phases (Clark, 1937; Matsuoka, 1935; Glick, 1937; Reid, 1937).

Bourne and Allen (1935), Matsuoka (1935), and Biswas and Das (1937) observed a parallelism between the rates of growth and the accumulation of ascorbic acid. Carter (1935) studied the effect of orange juice on the development of *Laminaria* gametophytes and found evidence of stimulation of growth. The results suggested that the effect might be due to the ascorbic acid contained in the orange juice. Havas (1935) and von Hausen (1935) found that ascorbic acid added to the nutrient medium in which seedlings were cultured caused an increase in growth. The latter author held the stimulative effect to be due specifically to ascorbic acid and not merely to the addition of organic substances to the nutrient medium, since a similar addition of glucose caused no increase in growth. Davies, Atkins, and Hudson (1937) investigated the influence of ascorbic acid on the regeneration of willow branches and on germination and found that at low concentrations it stimulates growth but at high concentrations retards it.

In contrast to the aforementioned results, experiments conducted by Kögl and Haagen-Smit (1936)

failed to show a stimulative effect on growth resulting from the addition of ascorbic acid to the nutrient medium in which excised pea embryos of several different types were cultured. A possible explanation for the contradictory results is to be found in similar tests with pea embryos conducted by Bonner and Bonner (1938). They found that the nine varieties employed could be grouped into two distinct classes: those which greatly increased their growth in response to added ascorbic acid and those which did not. Embryos of the former group had a low ascorbic acid content when grown without the addition of the vitamin to the culture medium, and those of the latter group had a comparatively high content when grown under the same conditions. The results showed that plants of the group which did not respond to ascorbic acid externally supplied could themselves synthesize a sufficient quantity to meet their requirements. The authors deemed it justifiable to conclude that ascorbic acid is quite as much a growth factor for one type of embryo as for the other.

As a result of his studies of the behavior of *Avena* coleoptiles from etiolated seedlings under experimental conditions, Clark (1937) came to the conclusion that ascorbic acid is not a cell-elongating substance. On the basis of evidence thus far presented in the literature, this conclusion does not appear justifiable. Except in completely dormant stages of plants such as dry seeds, living tissues of higher plants have not been found which are devoid of ascorbic acid. If a tissue fails to respond to ascorbic acid added to the culture medium, this may signify only that the concentration already present in the tissues is optimum and that no more is needed. In Clark's experiments it is evident that ascorbic acid was not a limiting factor in the bending of the coleoptile, but there is no proof to show that the acid which was normally present in the tissues themselves was not concerned in elongation when other essential growth factors not present in optimum concentrations were also supplied. Bonner and Bonner expressed a somewhat similar viewpoint in their discussion of some of the questions involved in determining whether or not some substance is essential for growth. Conclusive proof for or against the indispensability of ascorbic acid for cell elongation or for maturation will be difficult to obtain because at present no way is known by which tissues can be obtained which are devoid of it.

Bogart and Hughes (1935) found little difference in the vitamin C content of oats sprouted in light and in darkness. Virtanen and Eerola (1936) reported that during germination of peas the formation of vitamin C is independent of light but is greatly influenced by temperature. These observations are not in agreement with the findings of other investigators working with different types of plants (Heller, 1928; Veselkine et al., 1935a, 1935b; Clark, 1937; Matsuoka, 1935; Lee and Read, 1936; Lee, 1936; Glick, 1937). The greater quantity of ascorbic acid found by most workers in plants grown in light is

² Since this manuscript was prepared for publication a paper by Weier (Amer. Jour. Bot. 25 (7): 501-507, 1938) has appeared which bears on this question. The data appear to indicate that the author is justified in concluding that ascorbic acid is the substance responsible for the reduction of silver nitrate by the chloroplasts.

generally attributed to differences in the leaves in plants grown in light as compared to those grown in darkness. The chlorophyll-bearing tissues have been reported to be responsible for much if not most of the difference in the total quantity in plants grown in light and in darkness (Giroud, Ratsimanga, and Leblond, 1934, 1935; Clark, 1937; Randon, Giroud, and Leblond, 1935; Sugiura, 1936). Reid (1937) found a much greater content in the blades than in the petioles of cowpea leaves and, in a more detailed analysis of the blade itself, a higher content in the mesophyll than in the veins. Although a greater quantity of ascorbic acid is generally found in chlorophyll-bearing tissues than in the unpigmented parts, there is usually not a direct parallelism in the content of the two substances. Clark (1937) observed a gradient in the quantity of ascorbic acid in the coleoptiles of seedlings grown in darkness which corresponds to the chlorophyll gradient in plants grown in the light but noted that it did not depend upon chlorophyll for its presence or distribution.

containing clean #1 white quartz sand were placed in shallow graniteware pans. The seeds were planted about 7 mm. below the surface and the sand was saturated with water, the excess being poured off. No more water was added until after about 40 hours. Part of the plants were grown in a greenhouse kept at approximately 29°C., and others were grown in darkness at the same temperature, controlled within a range of 1°C. The atmosphere of the dark room was maintained at a humidity of approximately 80 per cent. An electric fan was used to circulate the air in an effort to maintain uniform conditions in different parts of the room. The position of the cultures was changed each day in order to equalize any effects not adequately controlled by the fan. The maintenance of uniform environmental conditions was found to be very important.

At the end of each 24-hour period, two of the cultures were removed for use in the ascorbic acid tests. The plants were washed free of sand, care being taken not to injure the roots. The greenhouse plants were

TABLE 1. *Variability in ascorbic acid content of different samples of 3-day old seedlings grown in darkness at 29°C.*

A			B		
Weight of sample G.	Ascorbic acid per plant Mg.	Ascorbic acid Mg./G.	Weight of sample G.	Ascorbic acid per plant Mg.	Ascorbic acid Mg./G.
9.04	0.143	0.158	8.47	0.149	0.176
9.40	0.151	0.161	8.80	0.152	0.173
9.17	0.144	0.157	8.64	0.148	0.171
9.23	0.147	0.159	8.60	0.148	0.172
9.07	0.143	0.157	8.98	0.145	0.161
9.39	0.148	0.157	8.55	0.147	0.172
8.93	0.144	0.161	8.74	0.153	0.175
8.75	0.142	0.162	8.62	0.146	0.169
9.19	0.144	0.156	8.70	0.144	0.165
9.06	0.145	0.159	8.60	0.145	0.169
Av. 9.12	0.145	0.159	8.67	0.148	0.170

The investigations which have been cited have contributed much to our knowledge of the distribution of vitamin C in plant tissues and something concerning its evolution in the life history of the plant. Much more study, however, is required on the effect of environmental factors, such as the influence of variations in intensity and quality of light, temperature, moisture, oxygen supply, and composition of the nutrient medium. Light has been found to have the most profound effect of any of the environmental factors thus far tested. For this reason it has been chosen as the first subject of a series of studies now in progress.

METHODS.—Seeds of the Groit variety of cowpeas used in these tests were selected as to size and planted in half-gallon glazed earthenware crocks of the shallow type. A small hole was made in the bottom of each jar to furnish the necessary drainage. The jars

grown during November, because it was possible to control the temperature more accurately than then could be done at a time of year when light was more intense. No nutrient solutions were added in this set of experiments. In other tests conducted at approximately the same time of year there was no influence of added minerals on the ascorbic acid content of the plants during the first 15 days of growth. Soon after the end of the 15-day period, however, a stimulative effect of fertilizers was noticeable. Under conditions of greater light intensity, as in April, the addition of fertilizers caused an increase in the quantity of ascorbic acid in the plants somewhat earlier than in plants grown in November.

A small proportion of cowpea seeds, like those of most leguminous plants, have hard seed coats and are consequently retarded in germination. In most experiments there were sufficient plants in the two

cultures used in each set of tests to make unnecessary the use of any of the backward individuals.

Ascorbic acid determinations of the seedlings grown in darkness were made at the end of 24 hours and daily thereafter for 11 days. At the end of the twelfth day the tissues of the seedlings were losing their former turgidity and showed other evidences of deterioration. Seedlings from the group grown in the light were harvested at daily intervals for the first five days and subsequently on alternate days up to and including the fifteenth day, at which time the first set of compound leaves were unfolding. In most of the tests the plants were assorted into five uniform

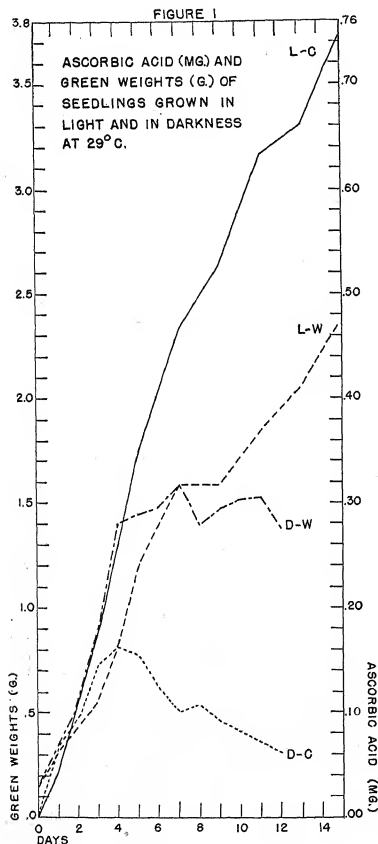
groups of ten specimens each, one of which was extracted with 2 per cent metaphosphoric acid in combination with 8 per cent acetic acid (Musulin and King, 1936), another with 2 per cent metaphosphoric acid in combination with 2.5 per cent sulphuric acid (Mack, 1936; Mack and Tressler, 1937), and a third with 10 per cent metaphosphoric acid (Fujita and Iwatake, 1935). The fourth and fifth groups were combined into one sample which was used in determining the ascorbic acid content of the various organs. Tests with the three types of extracting solutions were not made in all the experiments. The 10 per cent metaphosphoric acid solution was employed in the ascorbic acid determinations of the individual organs.

The tissues were thoroughly ground in a mortar, using some of the acid-extracting solution and a small quantity of acid-digested sand to facilitate proper disintegration of the cells. The solution containing the extracted ascorbic acid was then filtered and made up to volume and an aliquot used for determining the ascorbic acid content by titrating against a standardized solution of sodium 2, 6 dichlorophenol-indophenol.

The results obtained with the three types of extracting solutions agreed fairly closely, the differences being not greater than those probably due to variations in the samples.

Tests were made to determine the variability in ascorbic acid content of different lots of uniformly grown plants. Plants cultured in darkness at 29°C. were removed from the chambers at the end of three days. They were washed in distilled water and assorted into ten uniform lots of ten plants each. Each lot was weighed, placed in a Petri dish, and set in the ice box until ascorbic acid determinations could be made. Ten per cent metaphosphoric acid was used in the extractions. Ascorbic acid determinations were made of two groups of plants (A and B, table 1) treated in this way. Although the cultures were kept in the constant temperature chamber the same length of time, the plants of group A, which were planted one day earlier, were slightly larger than those of group B. Each figure in table 1 represents the value obtained from the plants of two cultures. The variations from the average fall within a range of about 5 per cent. Because of the difference in weight of the plants in the two groups, the ascorbic acid per gram of tissue was somewhat greater in the smaller plants constituting group B.

In some of the experiments with seedlings grown in darkness in which plants older than those in the above test were employed, somewhat greater variations between different lots of plants were found in total green weight and also in the relative weights of the organs. It was observed that plants which grew at somewhat different rates in their early stages, as those of groups A and B in table 1, tended later to show variations in relative weights of the different organs. The only known condition which might have caused these variations may have been a slight differ-



ence in the moisture content of the sand at the time of planting. From results of tests with cultures known to have slight differences in moisture content of the sand, it was observed that the plants grown in sand of higher moisture content tended to have longer, heavier hypocotyls and correspondingly shorter, lighter weight epicotyls. The roots also tended to weigh less. The effect of the higher moisture content of the

observed from the results of these experiments are (1) the striking effect of light on the accumulation of ascorbic acid in the seedlings, (2) a general parallelism between the rates of growth and accumulation of ascorbic acid in the different organs, and (3) the marked effect of light on the accumulation of ascorbic acid in the roots as well as in the leaves, an observation which has not been reported previously.

TABLE 2. Comparison of green weights and dry weights per seedling of different organs of plants grown—some in light, others in darkness. Age 12 days.

Light					
	Hypocotyls	Roots	Epicotyls	Primary leaves	Total
Green weights (g.)	0.210	1.391	0.221	0.547	2.37
Dry weights (g.)	0.0458	0.0842	0.0342	0.0846	0.249
Percentage moisture	78.2	93.9	84.5	84.5	89.5

Darkness					
	Hypocotyls	Roots	Epicotyls	Primary leaves	Total
Green weights (g.)	0.446	0.368	0.561	0.054	1.43
Dry weights (g.)	0.0204	0.0216	0.0274	0.0064	0.076
Percentage moisture	95.4	94.1	95.1	88.1	95.4

sand was apparently related to the amount of air available to the germinating seed, the more water the less air being present. Lower ascorbic acid values were invariably associated with conditions of less opportunity for air exchange. Due to the high degree of sensitivity of cowpea seedlings to aeration of the culture medium, the soil moisture factor was the most difficult to control with sufficient precision to produce results in one experiment exactly like those in another. Also, plants grown in light showed slight variations in one experiment as compared to another which were caused by differences in light intensity.

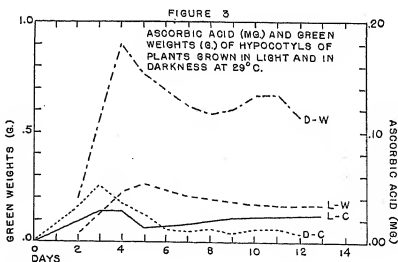
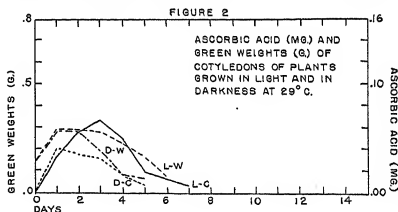
Available space in the dark room permitted approximately equal conditions of aeration, humidity, and temperature for not more than 28 cultures at one time. Sufficient plant material was thus not available for dry weight determinations of the various organs at different stages of growth. The results in general indicate a rather close relation between changes in green weight and ascorbic acid content of the tissues, but it is possible that a closer relation might be found from results calculated on a dry weight basis. Investigations to study certain aspects of this phase of the problem are now in progress.

Nitroprusside tests made to determine if free sulphhydryl groups were present in the seedling tissues all gave negative results.

RESULTS.—The total ascorbic acid and total green weight of the plants at different periods of development are presented graphically in figure 1. Figures 2-6 show similarly the green weights and ascorbic acid content of the various organs at different stages of development. The most outstanding feature to be

At the age of seven days plants grown in light contained more than four times as much and at the age of eleven days about nine times as much ascorbic acid as plants of corresponding age grown in darkness.

The striking differences in size and habit of growth of the two types of plants at the end of the experi-



mental period are those that are normally found in most types of dicotyledonous seedlings grown in light as compared to those grown in darkness. The difference in appearance of the two types of growth is shown in Plate 1. In another experiment a comparison was made of the green weights, dry weights, and percentage of moisture in the different organs of plants grown, some in light, others in darkness, the data of which are presented in table 2. The percentage of moisture in hypocotyls and epicotyls was found to be much greater in darkness than in light, a smaller difference was observed in the leaves, and very little difference in the roots.



Plate 1. Plant at right grown in darkness at 29°C. and the one at left grown in light at the same temperature.

The quantity of ascorbic acid in seedlings grown in darkness increased up to the fourth day, after which it soon diminished. This was also the time of almost complete exhaustion of the available food reserves in the cotyledons. Most of the gain in green weight in these seedlings also occurred within the first four days. As a consequence of the ability of the hypocotyls of plants grown in darkness to acquire a much higher water content than those of plants grown in light (table 2), the increase in green weights of the former plants was more rapid during the period of development of the hypocotyls—i.e., the first five days. The quantity of ascorbic acid per seedling in light and in darkness did not differ markedly until after the second day, at which time the primary leaves were developing noticeably.

The cotyledons of the plants grown in light became somewhat green, and their ascorbic acid content was greater than that of cotyledons of the plants grown in darkness. The cotyledons of the plants in the light shrunk less rapidly, probably because of the synthesis of organic compounds under the influence of light resulting in less rapid withdrawal of the stored reserves (Yocum, 1925). In darkness the cotyledons attained their maximum quantity of ascorbic acid by the end of 24 hours, whereas in the light the quantity increased up to the third day.

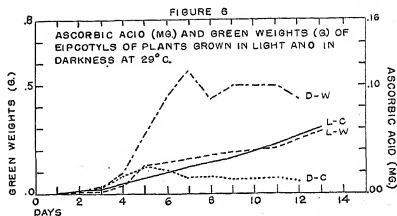
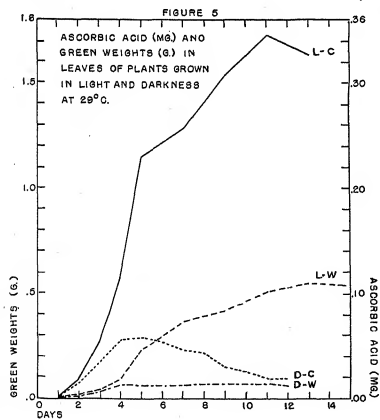
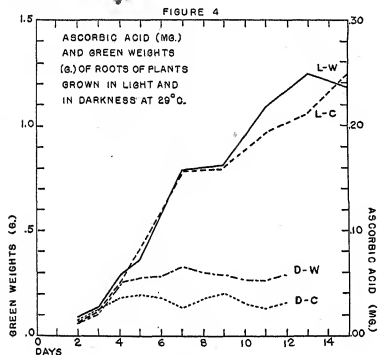
During the period of elongation of the hypocotyls their increase in green weight and ascorbic acid were both greater in darkness than in light. On the third day, for example, hypocotyls of plants grown in darkness weighed more than four times as much and contained almost twice as much ascorbic acid as those of plants grown in light. When the hypocotyls had attained full length under the two conditions, those of plants grown in light ranged from 4 to 5 cm. in length, whereas those of plants grown in darkness attained a length of 10 to 15 cm. The relatively large quantity of ascorbic acid found in the hypocotyls in the early stages of growth was undoubtedly a consequence of translocation from the cotyledons. As the tissue of the plants in darkness expanded, the percentage content of ascorbic acid diminished (table 3). In the hypocotyls of plants grown in the light the percentage decreased up to the fifth day and then gradually increased, the increase being possibly a result of translocation of the product newly synthesized in the leaves.

From the data presented in figure 5 it is evident that the very rapid increase in total ascorbic acid from the second to the fifth days in seedlings grown in light was brought about largely by the development of the primary leaves. The roots of these plants also exhibited a large increase both in growth and in ascorbic acid. The difference in quantity of the vitamin in the roots in light and in darkness was also indirectly related to the effect of light on the leaves; that is, either some of the ascorbic acid synthesized in the leaves in light was transported to the roots, or some of the carbohydrates synthesized by the leaves were transported to the roots and were there converted into ascorbic acid. Possibly the increase occurred by both of these methods. The ascorbic acid accumulated rapidly during the period of expansion of the leaf, and when full size was attained there usually was no further increase apparent unless the light or nutritive conditions of the plants were changed. This, however, does not necessarily signify that the leaves ceased to form ascorbic acid after growth was completed. It is possible that they continued the synthesis and translocated or in some way utilized the excess.

The leaves remained very small in darkness but had a high content of ascorbic acid in proportion to their weight and size. The average area per plant of the primary leaf blades from nine-day old plants grown in the light was 30.2 cm.² and in darkness 3.38 cm.². The former contained 0.0101 mgms. of

ascorbic acid per square centimeter of leaf surface and the latter 0.0087. In other tests conducted in light and in darkness at 22°C. the leaf areas were approximately the same as those of the corresponding group at the higher temperature. The quantities of ascorbic acid per square centimeter of surface were approximately equal in light and in darkness, being 0.0139 mg. in the light and 0.0138 in darkness. It appears from these data that if ascorbic acid is required as a structural material for leaf growth, the failure of the leaves in darkness to expand further at the 22°C. temperature cannot be due to lack of this substance but to an inadequate supply of some other substance, for the formation of which light is necessary. Ascorbic acid, however, may have a somewhat indirect relationship to leaf growth. A certain concentration per unit area of surface may be necessary as a physico-chemical conditioner of the cells. Growth

could then occur as long as the concentration tended to be greater than this essential level. A higher concentration of ascorbic acid per unit area of leaf surface was, in fact, found during the period of expansion of the blade. A more detailed description and discussion of these results will be given in a future publication.



sion of the blade. A more detailed description and discussion of these results will be given in a future publication.

There appeared to be no increase in size of the mesophyll areas of plants grown in darkness after the fourth to fifth days, although the leaves as a whole continued to gain in weight up to the ninth day. There was a decline in ascorbic acid after the fifth day. The loss in ascorbic acid in the leaves and also in the entire plants during the later phases of growth may be due to utilization of the acid in growth. It is also possible that the loss is due to decomposition. This is a question undoubtedly of considerable importance in the economy of the plant, but at present there is no convincing evidence in support of either supposition.

There appeared to be a close correlation between growth and the ascorbic acid content of the roots in plants grown in light and in darkness, although the size of the root system and the total quantity of ascorbic acid contained in it were very much greater in the plants kept in light. This difference was largely a result of the much more extensive branching in the latter plants. The contrast in appearance of the two types of root systems is well illustrated in the photographs shown in Plate 1.

The epicotyls developed relatively little until after the fourth day, when rapid growth occurred. The expansion continued until the seventh day in the plants kept in darkness, but in those exposed to light it was continuous until the termination of the experiment. The maximum accumulation of ascorbic acid in the epicotyls was found on the fifth day in darkness. The values shown in figure 6 for the epicotyls of plants grown in light include the buds of the first compound leaves. Since these were not developed in the early part of the experimental period, the values up to and including the fifth day represent stem tissue almost exclusively as did those of the plants grown in darkness. In these early phases of development the epicotyls of the plants kept in darkness grew faster and also contained more ascorbic acid than those kept in the light. On the fifth day the epicotyls of the plants in darkness had an average

TABLE 3. Ascorbic acid (mg.) per gram of tissue in seedlings grown at 20°C.—some in light, others in darkness.

Age (days)	Light					Entire seedling	Darkness					Entire seedling
	Cotyledons	Hypocotyls	Roots	Primary leaves	Epicotyls		Cotyledons	Hypocotyls	Roots	Primary leaves	Epicotyls	
1	0.108	0.404	0.285	1.000	0.136	0.143	0.357	0.217	1.025	0.833	0.193	
2	0.189			0.966	0.126	0.159		0.450	0.175			
3	0.241			0.947	0.164	0.095		0.928	0.161			
4	0.226	0.293	0.235	1.337	0.322	0.207	0.039	0.143	0.887	0.460	0.115	
5	0.060	0.123	0.216	0.980	0.283	0.317	0.033	0.144	0.984	0.089	0.107	
6	—	0.051	0.175	—	0.109	0.090	0.017	0.126	0.868	0.049	0.082	
7	0.084	0.084	0.205	0.701	0.159	0.294	0.017	0.081	0.693	0.031	0.061	
8	—	—	—	—	—	—	0.022	0.114	0.624	0.041	0.078	
9	—	0.121	0.204	0.739	0.170	0.331	0.013	0.140	0.388	0.026	0.062	
10	—	—	—	—	—	—	0.018	0.114	0.369	0.027	0.054	
11	—	0.145	0.226	0.687	0.212	0.343	0.017	0.100	0.248	0.030	0.017	
12	—	—	—	—	—	—	0.014	0.106	0.142	0.027	0.044	
13	—	0.142	0.238	0.596	0.212	0.322	—	—	—	—	—	
14	—	—	—	—	—	—	—	—	—	—	—	
15	—	0.154	0.187	0.608	0.391	0.316	—	—	—	—	—	

length of 11.5 cm. and those in light only 4.3 cm. Epicotyls of the former plants contained 95.12 per cent moisture, whereas the latter contained only 84.52 per cent. The growth and ascorbic acid relationships of the epicotyls of plants grown in light as compared to plants grown in darkness are thus similar to those of the hypocotyls. As in the hypocotyls, there was also a relatively high concentration of the vitamin in the young epicotyls.

In some of the experiments the epicotyls were divided into apical and basal portions. A higher percentage content of ascorbic acid was found in the apical segments. For example, the upper 4 cm. of the epicotyls of the eight-day stage had 0.065 mg. per gram, whereas the lower or older portion contained only 0.020 mg. per gram. A somewhat similar gradient was found in the plants grown in the light. In the latter plants there was a similar gradient in the distribution of chlorophyll and chloroplasts in the basal and apical portions of the epicotyls, the latter having a considerably higher content. This gradient in distribution in the stem in relation to chlorophyll is in accord with the observation of Clark (1937) that the regional distribution of ascorbic acid in plants grown in darkness corresponds to that of chlorophyll of plants grown in light.

The observations made in these experiments point to the conclusion that ascorbic acid distribution tends to parallel that of the chloroplasts, but no attempt has been made in this work to determine whether or not it is contained within them. In experiments which will be described more fully in a later publication there is abundant evidence that no essential parallelism exists between the chlorophyll and ascorbic acid contents of the tissues. Leaves may have a very low chlorophyll but high ascorbic acid content, and they may also have a very high chlorophyll content but a comparatively small quantity of ascorbic acid.

It appears probable that the ascorbic acid which was found in the seedlings germinated in darkness developed in the cotyledons and was translocated into the growing organs, but the possibility should not be overlooked that at least part of the acid may be formed from some precursor within the confines of each organ as it grows.

The chemical nature of the precursor is of great interest, but comparatively little is known about it. It has been generally assumed and there has been some experimental evidence to show (Ray, 1933) that a hexose such as glucose can be converted into ascorbic acid by the seedling during the process of germination. Tests were conducted to determine if this is true of cowpea plants. Seedlings were grown in sand and kept in darkness at 27°C. until three days old. They were then transferred to pint jars containing distilled water. After being allowed to grow in the water for five days, part of them were placed in a 0.5 per cent glucose solution, and others were kept in distilled water. The solutions were changed twice daily. After being in the solutions for four days, the

seedlings were weighed and ascorbic acid determinations made. The results (table 4) show that there was a very definite increase in ascorbic acid as a result of the availability of additional carbohydrate. There was no increase in elongation of either stems or roots, however, as a consequence of the access to additional carbohydrate. The stems were considerably tougher to section, indicating undoubtedly an effect of the sugar on cell wall thickening.¹

TABLE 4. *Effect of glucose in culture medium on the ascorbic acid content of seedlings grown in darkness at 27°C.*

		No glucose	0.5% glucose
Green weights	1	13.57	13.80
	2	12.90	12.22
Total ascorbic acid	1	0.805	1.518
	2	0.843	1.311
Ascorbic acid mg./g.	1	0.059	0.110
	2	0.065	0.107

Much of the growth of the epicotyls in darkness occurred between the fifth and seventh days, when there was a rapid decline in the total quantity of ascorbic acid contained within the epicotyls. The concentration of available carbohydrates was sufficiently high in the region of elongation of the epicotyl to permit the expansion processes to continue but apparently was not high enough to provide for any apparent increase in total ascorbic acid. It is possible, of course, that ascorbic acid may have been synthesized and then used in some way in elongation or maturation. In a number of instances it has been observed that plants grown in darkness which were increasing in size faster than others similarly grown tended to have slightly less ascorbic acid per plant. The difference in content may constitute a fraction that was being used in growth, but, as previously stated, there is, as yet, no convincing proof that the plant employs ascorbic acid as a structural material.

The practical application of the results of the experiments herein described is to be found in their relation to problems of human nutrition, since man is directly dependent upon plants for most of his supply of vitamin C. This is true also of several types of animals—namely, swine, monkeys, and guinea pigs. No other environmental factor has been found to have an effect upon ascorbic acid synthesis by the plant which in magnitude approaches that of light. Fruits and vegetables grown in deeply shaded areas or during prolonged periods of cloudy weather would, according to these and other, as yet unpublished, results, tend to have a lower supply of the vitamin than those grown in full sunlight.

SUMMARY

Light has a remarkable effect on the accumulation of ascorbic acid during germination and early stages of growth. Seven-day-old plants (the stage of maxi-

mum green weight of plants grown in darkness) grown in light contained more than four times as much and at the age of eleven days about nine times as much ascorbic acid as seedlings of corresponding ages grown in darkness.

A rough parallelism was found between growth and ascorbic acid content of all organs both in light and in darkness.

The quantity of ascorbic acid contained in seedlings grown in darkness increased up to the fourth day and decreased after the fifth day. Rapid increase in green weight occurred during the first four days, then lagged markedly for three days, following which there was a decline. In light, growth and ascorbic acid increases were continuous.

It is not known whether the loss of ascorbic acid in darkness was a result of destruction of the substance or of utilization in growth.

Ascorbic acid accumulated in the cotyledons of germinating seedlings and was undoubtedly translocated to other organs both in light and in darkness.

After the food reserves in the cotyledons were exhausted no further increase in ascorbic acid in seedlings grown in darkness was found, notwithstanding the fact that there was sufficient material within the plant to provide for most of the growth of the epicotyl.

The increased synthesis of ascorbic acid in the light was undoubtedly due, directly or indirectly, to the influence of light on the activity of the chloroplasts.

Ascorbic acid formed in the leaves in tissues containing chloroplasts was apparently translocated in part to other organs, but a high concentration was maintained in the leaf tissues.

Light appeared to be essential also for the synthesis of a substance other than ascorbic acid which is concerned with the expansion of the blade.

A higher content of ascorbic acid was found toward the apex of the stem than in the lower portion. The quantitative distribution in the stem is similar to that of chloroplasts in plants grown in the light.

When additional carbohydrate in the form of glucose was supplied to the seedlings at the time when the reserves in the cotyledons were nearing depletion, an increase in ascorbic acid content of the plants was found. This may indicate a fairly direct conversion of glucose to ascorbic acid but not necessarily so.

Very great differences were found in the moisture content of the stems, much less in the leaves, and very little difference in the roots of cowpea plants grown in light as compared to those grown in darkness.

The importance of light for ascorbic acid synthesis has a bearing on problems of considerable importance to human welfare. Fruits and vegetables grown under conditions of low light intensity would, according to these and other unpublished results, tend to have lower vitamin C values than those developed under conditions of greater illumination.

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THE ACTION OF ETHYLENE ON PLANT GROWTH¹

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DURING THE past sixty years a number of workers have noted the curious effects which ethylene produces on plants. The most noticeable of these effects are included in the "triple response" of etiolated pea seedlings to ethylene described by Crocker, Knight, and Rose (1913). This response consists of reduction in rate of stem elongation, increase in stem diameter, and absence of normal geotropic response. These authors found their seedlings to be sensitive to ethylene in concentrations of less than one part per million parts of air. Furthermore, they found no other gas which produced a similar response except in much higher concentrations.

The abnormal geotropic response had previously been studied by Neljubow (1911), who considered it to be transverse geotropism, and Richter (1910) who considered it as ageotropism. Van der Laan (1934) was the first to consider the possibility that ethylene acts on the auxin in the plant. If normal *Vicia faba* seedlings were placed in a horizontal position, auxin was concentrated on the lower side of the stem, as described by Dolk (1930). In ethylene-treated plants, however, this redistribution failed to occur. The present author has noted that, while ethylene-treated *Pisum* seedlings fail to respond geotropically, this is not true for seedlings of *Zea mays* and *Avena sativa*.

Ethylene also affects the geotropic response of other parts of plants. It causes leaf epinasty in many species but not in all (Crocker, Zimmerman, and Hitchcock, 1932). It also affects the geotropic response of roots (Molisch, 1884; Zimmerman and Hitchcock, 1933).

The other two constituents of the "triple response"—reduced longitudinal growth and increased growth in diameter—are the ones to be considered in this paper. Van der Laan (1934) has also considered these and has come to the conclusion that ethylene causes a decrease in auxin production which in turn causes decreased elongation of the stem. He considered the increase in stem diameter to be a result of the inability of the stem to grow in length—a theory which now fails to agree with available facts.

One other effect of ethylene on plant growth should be mentioned here. Ethylene treatments have been shown by Zimmerman and Hitchcock (1933) to cause initiation of adventitious roots and outgrowth of pre-existing root primordia on the stems of many species of plants and in some cases on their leaves and roots.

Within recent years experiments have been done which show that ethylene is produced in plant tissue.

Elmer (1932) showed that apples give off a gas which causes potato sprouts to become abnormally short and thick. Botjes (1933) suggested that this gas was ethylene. Cane (1934) obtained a gas from apples which he chemically identified as ethylene. Elmer (1936) showed that apples give off a gas which causes a typical ethylene response in potatoes and tomatoes. Denny and Miller (1935) and Denny (1935) obtained a gas causing epinasty in potato leaves from a large number of leaves, roots, tubers, and parts of flowers. According to Denny (1936), more of this gas was produced by horizontally placed tomato stems than by ones placed in a vertical position. Zimmerman and Wilcoxon (1935) found that addition of heteroauxin increased the capacity of tomato plants to give off this gas. The gas obtained by these workers was probably ethylene, though it was not chemically identified as such.

The fact that ethylene is produced by plants and that it causes growth responses in plants has led to the suggestion by Crocker, Hitchcock, and Zimmerman (1935) that ethylene itself is to be considered as a plant growth hormone. This is discussed further at the end of this paper.

EXPERIMENTAL METHODS.—Experimental material.—The plants used in these experiments were etiolated seedlings of pea (*Pisum sativum*, var. Alaska), corn (*Zea mays*), and oat (*Avena sativa*). The first two were grown in moist sand, in a dark room which was kept at a temperature of about 24°C. and a relative humidity of 80 to 90 per cent. The oat seedlings were grown under the same conditions, but in water instead of moist sand.

Application of ethylene to plants.—Plants were treated with ethylene either in a large incubator of 180 liters' capacity or in glass desiccator jars of about 10 liters' capacity. In either case the plants were kept in the dark during treatment, and the temperature was 24°C. For relatively high ethylene concentrations the ethylene was added through a gas burette of appropriate size. For low ethylene concentrations in the small desiccators, it was found practical to add a measured volume of a saturated solution of ethylene in water. When the solution came in contact with the air in the desiccator, the ethylene of course quickly evaporated. Since one volume of water dissolves about 0.11 volumes of gaseous ethylene, it may be seen that this method was much easier than it would have been to measure extremely small amounts of gaseous ethylene. In all cases, ethylene was added in the amount necessary

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to give the concentration indicated in the description of the experiment.

Application of heteroauxin to plants.—In some experiments heteroauxin was dissolved in lanoline and applied as a paste, according to the method of Laibach (1935). Otherwise blocks of 1.5 per cent agar were soaked in a heteroauxin solution and applied to the plant, as described by Went and Thimann (1937). In all cases, heteroauxin (indole-3-acetic acid) was used for such applications.

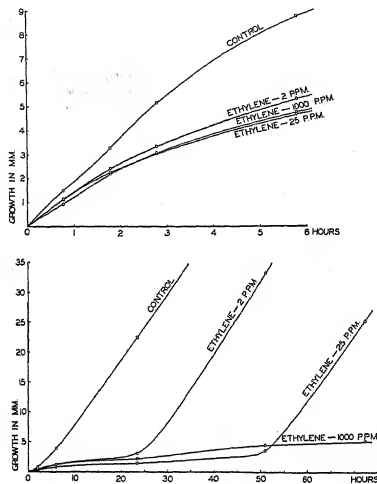


Fig. 1 (above). Effect of ethylene on growth of oat seedlings. The seedlings were about 2 cm. high at the beginning of the experiment.

Fig. 2 (below). Effect of ethylene on growth of pea seedlings. The seedlings averaged about 2 cm. high at the beginning of the experiment.

Tests for auxin.—The test most often used in these experiments is the standard *Avena* test described by Went (1928; Went and Thimann, 1937). The essential features of this test are as follows: The growing out coleoptile is decapitated, and the agar block to be tested is applied to one side of the stump. The auxin from the block passes into one side of the coleoptile, causing growth on that side with consequent curving, which is proportional to the amount of auxin in the block. In these experiments, large agar blocks ($6 \times 8 \times 1$ mm.) were used for collecting the auxin. They were then cut into twelve small blocks, each of which was applied to a plant. The amount of auxin in the block is stated in terms of the number of degrees of curvature it produces in the *Avena* test. Since heteroauxin and the auxins

occurring under natural conditions in plants (auxin a, auxin b) have almost the same physiological properties, this test fails to distinguish between these substances. Consequently, in describing the results of these experiments, the term "auxin" is used as a collective term indicating any or all auxins.

EXPERIMENTAL RESULTS.—**Effect of ethylene on auxin in vitro.**—In several experiments agar blocks containing auxin were treated with ethylene in concentrations varying between .01 per cent and 100 per cent, for periods of from one to four hours. In no case was a significant difference detected between treated blocks and controls which were not treated. This is in agreement with similar experiments of van der Laan (1934).

Effects of ethylene on longitudinal growth in oat seedlings.—As has been mentioned, ethylene treatment causes a marked reduction in the growth rate of the oat coleoptile. The magnitude of this reduction is independent within wide limits of the ethylene concentration. This is clearly shown in the experiment represented in figure 1, in which ethylene concentrations of 2 p.p.m. (parts per million), 25 p.p.m., and 1000 p.p.m. (0.1 per cent) caused practically the same growth reduction during the first six hours of treatment. In previous experiments (Michener, 1935), it was shown that ethylene inhibits growth to the same degree even though its concentration is raised to 20,000 p.p.m. (2 per cent).

If this growth reduction is due to action of ethylene on auxin, the ethylene must act on one or more of the following processes: (1) auxin production, (2) auxin transport, (3) auxin destruction, (4) sensitivity of the plant to auxin. (All but (3) were suggested by van der Laan, 1934).

The effect of ethylene on production was determined as follows: Plants were placed in 0.01 per cent ethylene (with controls in air) for several hours. Then the tip (about 1.5 mm. long) was removed from each plant and placed on wet filter paper for 40 minutes to 2 hours for the purpose of removing oxidative enzymes liberated at the cut surface, which might otherwise destroy part of the auxin coming out of the tip.² After this the tips were placed on agar blocks (24 tips to the block), and the blocks with ethylene-treated tips were again placed in ethylene. After two hours the tips were removed, and the blocks were tested for auxin by the standard *Avena* test. It may be seen (table 1) that there is no difference in auxin production between ethylene-treated and normal plants.

Since this is the exact opposite of the result obtained by van der Laan (1934) for similar experiments, the experiment was repeated, using the same experimental procedure that he used. This differed from that described above by the omission of washing of the cut surfaces on wet filter paper. As shown in table 1, this gave results similar to his—more auxin

²The work of Thimann (1934) and van Overbeek (1935) indicates that oxidative enzymes are responsible for destruction of auxin.

entered the agar blocks from the control plants than from the treated plants.

These experiments show that auxin is more rapidly destroyed at the basal cut surface in tips from ethylene-treated plants than in tips from normal plants. Since this difference can be eliminated by washing the surface, however, it appears that ethylene increases destruction only on the cut surface and that in intact plants ethylene does not affect the production of auxin.

would have resulted in a decrease in the total amount of auxin recovered from the two blocks.

Auxin destruction was also measured by the method of van Overbeek (1935), in which sections from previously decapitated (and therefore relatively auxin-free) coleoptiles were placed on agar blocks containing a known amount of auxin. After two hours the agar blocks were tested for auxin. In all cases sections from ethylene-treated plants caused the same amount of destruction as those from normal plants. It is

TABLE 1. *Effect of ethylene on auxin production in oat seedlings.*

Experiment no.	Time in ethylene hours	Auxin produced (degrees curvature)	
		Ethylene	Pure air
A. Cut surface washed on wet filter paper:			
1	3½	4.8 ± 0.6 ^a	4.9 ± 0.8
2	4	7.1 ± 0.5	6.2 ± 0.3
3	13	5.4 ± 0.4	5.1 ± 0.5
4	14	4.2 ± 0.3	5.6 ± 0.4
5	16	4.5 ± 0.2	4.7 ± 0.3
6	16	4.0 ± 0.4	4.4 ± 0.6
Average		5.0 ± 0.2	5.1 ± 0.2
B. Cut surfaces not washed:			
7	16	9.1 ± 0.5	12.0 ± 0.7

^a Probable error is used as a measurement of precision.

The effect of ethylene on auxin transport in oat coleoptiles has been investigated by van der Laan (1934). His data are extensive and show no effect of ethylene on transport. Similar experiments by the author gave the same result. These experiments were carried out by cutting coleoptile sections from ethylene-treated and normal plants, placing them in upright position on pure agar blocks, and placing on

not known why these results disagree with those of the experiments on production, which indicated that ethylene does increase destruction on the cut surface of coleoptile tips. These experiments, however, also have failed to show any effect on destruction within the plant tissue. Therefore it may be concluded that the effect of ethylene on destruction is lacking or negligible in the intact plant.

TABLE 2. *Effect of ethylene on the sensitivity of the Avena test.*

Auxin concentration (mg./l.)	No. of test plants used	Treatment with 0.01% ethylene		Average curvature of plants (degrees)	Increase caused by ethylene (degrees)
		Pretreatment (before reaction) hours	Treatment (during reaction) hours		
0.1	12	Over 2½	1½	11.7	5.0
	156	Over 2½	0	11.1	4.4
	72	0	1½	7.7	1.0
	168	0	0	6.7	
0.5 (giving maximum angle)	12	4½	1½	30.2	6.5
	12	4½	0	30.1	6.4
	12	0	1½	24.1	0.4
	24	0	0	23.7	

top of them blocks containing auxin. After two hours, both top and bottom blocks were tested for auxin. In all cases, the final auxin concentration in both top and bottom blocks was the same in normal and in treated plants. This not only indicates that transport was not affected by ethylene, but that destruction was unaffected. An increase in destruction

The effect of ethylene on sensitivity of the oat plants to auxin was next considered. This was first investigated by observing the effect of ethylene on the standard *Avena* test. The test was carried out as usual except that the treated plants were placed in a large sealed jar containing ethylene, and the controls in a similar jar containing no ethylene. Eight

experiments of this nature were carried out at different times, all giving essentially the same result. The data are averaged and summarized in table 2. It may clearly be seen that ethylene treatment during the reaction to auxin (i.e., while the curvature is taking place) has no effect on sensitivity of the coleoptile to auxin. The same is true for short pretreatments preceding the application of auxin to the coleoptile. Surprisingly, however, pretreatments lasting two and a half hours or over cause an increase in the sensitivity of the plants to auxin.

The sensitivity of ethylene-treated and normal coleoptiles to auxin was also tested by the method of Bonner (1933) in which sections about 5 mm. long were cut out of the coleoptiles, measured, placed in auxin solution for about 15 hours, and measured again.

growing zone, it has been shown that ethylene does not affect production, transport, or destruction of auxin. Furthermore, ethylene treatment of intact plants actually increases the sensitivity of these plants to auxin, as shown either by the *Avena* test of Went or the section test of Bonner. This suggests that ethylene, in this case, is affecting some other factor having to do with growth. The existence of one such factor, termed "food factor," has been shown by Went (1935), and it is suggested that ethylene causes activation or accumulation of this or some other growth factor.

Related to this hypothesis is the work of Went (unpublished), showing that phenyl-butyric acid, when applied as a lanoline paste, causes a reduction in growth of intact oat coleoptiles to whose tops it is applied. Also coleoptile sections from plants treated

TABLE 3. *Effect of ethylene on growth of coleoptile sections.*

Experiment	Auxin concentration (mg./l.)	Treatment	Growth in mm. (average of 25 plants)	Change caused by ethylene
A. Ethylene treatment during experiment	0.06	Pure Air	1.18	21% decrease
	0.06	Ethylene	0.93	
	0.006	Pure Air	0.80	29% decrease
	0.006	Ethylene	0.57	
B. Plants pretreated with ethylene	0.03	Pure Air	0.56	116% increase
	0.03	Ethylene	1.21	
	0	Pure Air	0.10	280% increase
	0	Ethylene	0.38	

In the first experiment, plants were grown in pure air, and one 5 mm. section was cut from the top of each plant. These sections were measured and then divided into two groups. The first group was placed in auxin solutions which remained in pure air. The second group was placed in similar auxin solutions which were in equilibrium with an atmosphere containing 0.01 per cent ethylene. After about 15 hours, the sections were removed and measured again. It was found that the ethylene-treated sections grew slightly less than did the controls (table 3, Experiment A). As will be seen later, it is of interest to note that ethylene treatment did not increase the growth of sections which were cut before being treated with ethylene.

In the next experiment, intact plants were pretreated with 0.01 per cent ethylene for six hours before the experiment, after which one 5 mm. section was removed from each plant, placed in a solution containing heteroauxin, and measured after 24 hours. In this case the ethylene had the same effect as in the *Avena* test; that is, it greatly increased the sensitivity of the coleoptile sections to auxin (table 3, Experiment B).

It has been shown (from fig. 1) that ethylene exhibits a strong inhibitory action on growth of intact oat seedlings. Although this suggests immediately that it decreases the amount of auxin present in the

with this substance are more than normally sensitive to auxin. The effect of phenyl-butyric acid is thus similar to that of ethylene. This has been verified by further experiment in which plants were treated with ethylene, with phenyl-butyric acid, and with both substances. In all cases sections from these treated plants grew more than sections from untreated controls. Went has suggested that phenyl-butyric acid causes activation of food factor. Thus it not only affects growth in the same manner as ethylene, but it appears to act by means of the same mechanism. Cyclohexane-acetic acid was found by Went and by the author to have similar effects.

Effect of ethylene on longitudinal growth of pea seedlings.—As in the case of oats, the inhibiting action of ethylene on longitudinal growth-rate of peas does not depend on the concentration of ethylene, within the limits of concentration used in these experiments. As shown in figure 2, an ethylene concentration of 2 p.p.m. inhibited growth to about the same degree as did a concentration of 1000 p.p.m. (0.1 per cent). There are two differences to be noted, however, between pea and oat seedlings. First, ethylene has a much greater inhibiting effect on longitudinal growth of peas than on that of oats. Also, while the degree of growth-inhibition in pea seedlings does not vary with ethylene concentrations, the duration of such inhibition is much greater with higher

concentrations. This was not observed in the similar experiment with oat coleoptiles; but in that case it could not be expected, due to the short growth period of the oat coleoptile.

Experiments were also done on the relative efficacy of various ethylene concentrations in producing ageotropism. A concentration of 5 p.p.m. caused slight bending of the stems. Almost complete ageotropism was caused by 20 p.p.m. and by 100 p.p.m. In some cases a concentration of 1000 p.p.m. gave a stronger response. Again, therefore, it appears that a very low ethylene concentration gives or approaches a maximal response.

Tests were first made to determine the amount of auxin in various parts of the pea seedling. Plants were treated with 0.1 per cent ethylene for 5 to 18

tion of stem was cut from near the top and placed on agar blocks as described above. The blocks were tested and gave the following curvatures: Ethylene-treated plants $0.4^{\circ} \pm 0.4$; controls $3.8^{\circ} \pm 0.6$. Although the data represent only one experiment (24 plants), they show that much less auxin was extracted from the ethylene-treated plants than from the normal ones. Since each group of plants received the same amount of auxin, this indicates that ethylene must have affected destruction or transport of auxin.

Consequently destruction was investigated in the manner previously described for oat seedlings. Plants were treated with ethylene for 15 to 20 hours, after which sections were cut from the stems and placed on agar blocks containing a known amount of auxin. (12 sections were placed on each block.) Subse-

TABLE 4. Effect of ethylene on the amount of auxin present in pea seedlings.

Experiment no.	Time in ethylene hours	Auxin extracted (curvature in degrees)		Part of plant used
		Ethylene	Controls	
1 ^a	18	3.8	12.8	Tip
		2.6	4.7	Middle
		3.4	1.3	Base
2 ^a	14	2.0	16.5	Tip
		0.6	7.5	Middle
		-0.5	1.4	Base
3	4½	1.8	12.5	Tip
		1.7	9.3	Base
4	17	0.1	3.5	Tip
5	16	1.8	5.8	Tip (var. Alaska)
		0.5	5.0	Tip (var. Perfection)

^a Tests made with deseeded plants, by the method of Skoog (1937).

hours, after which tips and sections from the middle and base of the stems were cut and placed on wet filter paper for about 10 minutes, in an effort to remove auxin-destroying enzymes from the cut surface. They were then put on blocks of pure agar (12 tips or sections per block) for one hour. The blocks were then tested for auxin content by the *Avena* test. In every case, sections and tips about 1 cm. long were used.

These experiments (table 4) show that much less auxin was extracted from the ethylene-treated plants than from the corresponding parts of the normal plants. This decrease could be caused by an influence of ethylene on production, destruction, or possibly transport of auxin.

If ethylene affects only production of auxin, it should be possible to remove this effect by substituting an artificial source of auxin for the natural one. Accordingly, seedlings were decapitated, and to the cut surface was applied lanoline paste containing 0.02 per cent heteroauxin. Half an hour later the plants were placed in 0.1 per cent ethylene, where they remained for 3½ hours, after which a 1 cm. sec-

quently the blocks were tested for auxin by the *Avena* test. As shown in table 5, the sections varied greatly in different experiments in their power to destroy auxin, but in every experiment the ethylene-treated sections destroyed much more auxin than the normal ones.

Unfortunately these experiments do not show definitely whether ethylene increases destruction in intact tissue or only on cut surfaces. It is impossible to prevent destruction by washing the cut surfaces; but on the other hand, destruction is very rapid both in the normal and ethylene-treated plants, and the failure of washing to stop it cannot be considered as proof that it occurs elsewhere than at cut surfaces.

Attempts were made to measure transport in sections of ethylene-treated and normal plants, by the method described for oat seedlings. It was impossible to obtain reliable data, however, for the effects of auxin-destruction were very large compared to those of auxin transport. The data at hand, however, indicate that ethylene has no great effect on transport of auxin.

Investigations were also made of the effect of ethylene on the pea test (van Overbeek and Went, 1937). For this test, pea seedlings are grown to a height of about 10 cm., decapitated, and the top 3 cm. section is removed. If this is split longitudinally and put in water, the resulting expansion of the pith causes the two halves of the stem to curve outward. If, however, they are placed in auxin solution, the ends will curve inward after several hours, and the inward curvature is proportional to the logarithm of the auxin concentration.

ethylene treatment during the reaction to auxin had no effect, while pretreatment before the plants were cut caused a reduction of about 45 per cent in the curvature.

This experiment also shows that treatment with ethylene alone, without auxin, fails to produce significant curvature. Thus it is shown that ethylene fails completely to stimulate growth in the absence of auxin.

The reduction of curvature caused by ethylene in the pea test was somewhat unexpected, since ethylene

TABLE 5. *Effect of destruction of auxin in pea seedlings.*

Experiment	Ethylene concentration	Auxin concentration in agar block (degrees curvature)		
		Original	Final	
			Ethylene	Air
1	0.01%	19.0	8.9	13.2
2	"	25.4	7.6	13.0
3	"	19.0	3.5	9.3
4	"	25.4	1.0	4.8
5	"	17.4	2.8	6.4
6	"	15.2	2.6	11.8
7	"	17.3	3.2	10.9
8	0.0005%	17.3	4.3	12.3
Average		19.5	4.2	10.2

For such an experiment, four groups of plants were used, as follows: (1) controls, (2) treated with 0.05 per cent ethylene during the experiment (i.e., while the sections were in auxin solution), (3) treated with 0.05 per cent ethylene for seven hours before the experiment, (4) treated with ethylene both before and

increases the sensitivity of oat seedlings to auxin. In view of the high rate of auxin destruction in peas and the fact that this destruction is increased by ethylene, these data should not be considered as evidence against the hypothesis that ethylene causes accumulation or activation of food factor.

TABLE 6. *Effect of ethylene upon the pea test.*

	Water	Solution used	
		Heteroauxin 0.6 mg./l.	Heteroauxin 3.0 mg./l.
1. Control (air)	2°	107° ± 7	304° ± 12
2. Ethylene during experiment	2°	84° ± 8	277° ± 14
3. Ethylene before experiment	0°	49° ± 7	145° ± 15
4. Ethylene before and during the experiment	0°	61° ± 4	155° ± 11
Average of 1 & 2	2°	96°	291°
Average of 3 & 4	0°	55°	150°
Reduction caused by ethylene treatment before experiment		43%	48%

during the experiment. The curvatures were measured fourteen hours after the plants were placed in the solution. To eliminate possible error due to slow diffusion of ethylene into the auxin solutions in groups (2) and (4), solutions were used which contained sufficient dissolved ethylene to be in equilibrium with the 0.05 per cent of gaseous ethylene in the atmosphere in which they were placed. From the results of this experiment (table 6) it may be seen that

Another method was developed for testing the effect of ethylene on sensitivity of pea stems to auxin without splitting the stems. The liberation of destructive enzymes from a large area of cut surface was thus eliminated. To do this, 3 cm. sections of pea stems were placed in such a position that ethylene could be applied to one side but not the other. This was accomplished by cutting slits 3 cm. long and about 1 mm. wide in a thin metal plate which was then

coated with paraffin. Sections of pea stem 3 cm. long were cut and placed in water for 3 hours, after which they were placed lengthwise in the slits and fastened in with gelatin. Wet filter paper was placed at their ends to supply water. Next, the metal plate was sealed with vaseline to the open end of a battery jar (placed so that the sections were in an upright position), into which enough ethylene was admitted to make a concentration of 0.1 per cent. A gentle current of moist air was blown onto the outside with a fan in order to remove ethylene that diffused through the sections, thus holding the ethylene concentration as low as possible on the "air" side. After three hours the stems were removed and placed in a solution containing 0.1 mg. heteroauxin per liter. At that time they were straight and turgid. After about 15 hours they were removed, photographed; and their curvatures measured.

If the high ethylene concentration on the inside causes a greater increase in sensitivity to auxin than the low concentration on the outside, the auxin will cause the stems to bend away from the side which

both plants were examined microscopically. Neither in this case nor by macroscopic examination was any difference revealed between ethylene- and auxin-induced swellings. The swellings in pea seedlings were similar to those described by Czaja. In corn they had the same general characteristics but lacked root primordia.

In all cases it was found that swelling was associated with a decrease in longitudinal growth. This is to be expected from the nature of the swellings, as the cells become rounded and remain much shorter than normal. This also explains, in part, the previously described inhibiting action of ethylene on elongation of pea stems. The ethylene appears also to have some other effect on elongation, however, for it inhibits elongation when in concentrations too low to cause formation of swellings, as well as in oats in which such swellings do not occur.

Necessity of auxin for swellings.—Decapitated pea seedlings were treated with heteroauxin (0.02 per cent lanoline paste) and with 0.1 per cent ethylene for 6 days, as shown in table 7. From the results it may

TABLE 7. Necessity of auxin for swellings.

	Treatment of plants	Swellings
Intact plants	Ethylene or auxin	Large swellings
Decapitated plants	No ethylene, no auxin	No swellings
	Ethylene alone	Very slight swellings
	Auxin alone	Large swellings
	Ethylene and auxin	Large swellings

was treated with ethylene. This was, in fact, found to be the case, for two experiments comprising thirty-one separate plants gave an average curvature of 9.0 ± 1.9 degrees away from the ethylene-treated side. Though there was considerable variability among individual plants, the probable error of 1.9 shows the results to be significant.

This result may easily be interpreted in the same way as were the experiments on sensitivity of ethylene treated oat coleoptiles to auxin. If the ethylene causes a greater accumulation of food factor on the "ethylene" side than on the "air" side, the "ethylene" side will be more sensitive to auxin, and the observed curvature will result when the stems are placed in an auxin solution.

Ethylene-induced enlargement of stems.—As previously mentioned, several authors have noted that ethylene treatment often induces swelling or thickening of the stems of plants. Similar swellings are produced by application of heteroauxin in high concentration (Czaja, 1935; and others). As described by Czaja, the cells in auxin-induced swellings fail to elongate properly but round out and become nearly isodiametric. This change in shape of the cells is followed by increased cell division. In these experiments, both auxin- and ethylene-induced swellings were observed in etiolated pea seedlings and etiolated corn seedlings.² Free-hand sections of swellings from

be seen that ethylene failed to induce swelling formation only in the case where the natural source of auxin was removed by decapitation, and no auxin was added artificially. From this it may be concluded that auxin (in concentrations occurring naturally) is necessary for formation of swellings.

Necessity of roots for auxin-induced swellings.—Went (1935) has shown that formation of auxin-swellings is dependent on the presence of roots. If "de-rooted" pea seedlings are treated with auxin paste, the resulting swellings are very small or totally lacking.

In the experiments reported here it has been shown in two ways that formation of these swellings is not dependent upon root pressure. (1) If the roots are removed, root pressure certainly disappears almost immediately. Yet if the roots are removed and auxin applied within a short time, swellings form which are indistinguishable from those formed by plants with roots. If, however, the roots are removed two or three days before treatment of the plants with auxin, no swellings appear. (2) A more direct proof is furnished by leaving the roots out of water (in moist air) but supplying water to the plant through a slit cut in the stem just above the seed. It was found that both ethylene- and auxin-induced swellings on these plants were equal in size to those on normal plants which were not so treated.

² These swellings do not form in oat seedlings.

TABLE 8. *Effect of ethylene and auxin on stem swellings.*

		Pea seedlings				Corn seedlings	
		Stem diameter mm.	Percentage increase caused by treatment	Average wet weight of 3 cm. section mg.	Percentage increase in weight caused by treatment	Mesocotyl diameter mm.	Percentage increase caused by treatment
With roots	Pure Air	2.01 \pm .03		82		2.71 \pm .04	
	Ethylene	4.81 \pm .11	139	278	238	3.94 \pm .03	45
	Auxin	3.92 \pm .14	94	246	200	3.29 \pm .04	21
	Ethylene and auxin	5.29 \pm .09	163	330	303	3.35 \pm .04	24
Without roots	Pure Air	2.01 \pm .03		81		3.02 \pm .02	
	Ethylene	2.24 \pm .07	11	106	31	3.29 \pm .09	9
	Auxin	3.08 \pm .10	52	142	73	3.26 \pm .08	8
	Ethylene and auxin	2.46 \pm .10	22	100	22	3.31 \pm .10	9

Necessity of roots for ethylene-induced swellings.—Since auxin in high concentration will not induce swellings in de-rooted plants, it was desirable to know whether roots were necessary also for formation of ethylene-induced swellings. For this purpose pea seedlings were grown on wet filter paper in the dark to a height of about 3 cm., after which half of them were de-rooted and placed in vials to hold them upright. After two days each group was divided into four sub-groups (each containing 10 plants), which were treated respectively with ethylene, with hetero-auxin, with both substances, and with neither one. Lanoline paste containing 0.02 per cent heteroauxin was applied to all sides of the 5 mm. section of stem immediately below the sharp bend which always appears near the tip. Ethylene was applied in a concentration of 0.1 per cent for two days.

After another two days the diameter of each plant was measured at the point where swelling occurs with a low power microscope and ocular micrometer. Also, a 3 cm. section was cut from the stem at this point and weighed. The data, represented in table 8, leave no doubt that the capacity of the plants to form swellings is greatly reduced within two days after the roots are removed.

A similar experiment was done with corn seedlings. Here the swelling forms at the intercalary meristem near the top of the mesocotyl or first internode. The diameter of the largest part of the mesocotyl was therefore measured, as well as that of the coleoptile. Otherwise the experimental procedure was the same as with pea seedlings (except that the plants were only 1 or 2 cm. high, and the lanoline paste contained auxin in a concentration of 0.2 per cent). Also the experiments with normal and with de-rooted plants were not done simultaneously. Therefore the two groups of data cannot be directly compared, though they are both presented in table 8. It may be seen from the data that here, as in the case of peas, ethylene, auxin, or both together caused a large increase in mesocotyl diameter in the plants with roots.

In the plants without roots this increase is small and is not statistically significant. The method of action of ethylene in the production of swellings will be considered below.

DISCUSSION.—*Entry of ethylene into a plant.*—It seemed possible that ethylene, when applied in low concentrations, might require considerable time for entry into a plant. It was calculated however, (see Jacobs, 1935) that if ethylene were applied in a concentration of 0.1 per cent to a cylinder of water 1.5 mm. in diameter, it would reach a physiologically effective concentration at the center of the cylinder in about a second. As ethylene can pass more rapidly through the air spaces of the stem than through water, and as neither the cell walls nor the lipid layers of the cells can be expected to offer great resistance to the movement of ethylene, which is a hydrocarbon of small molecular size, it seems impossible that it will fail to penetrate a stem of similar size within a few minutes.

Concentration of ethylene.—In most of the experiments described here, the ethylene concentration was far above the minimal concentration necessary to affect growth, but low enough so it did not have toxic effects. This concentration (0.01 per cent to 0.1 per cent) produces all the effects on growth commonly attributed to ethylene and does not produce any effects which have not been previously attributed to ethylene. Consequently there seems to be no reason to believe that ethylene in this concentration is qualitatively different in its action than in much lower concentrations. These relatively high concentrations have been used here because their effects are, in some cases, more definite and therefore more easy to investigate.

Action of ethylene on elongation.—According to van der Laan (1934), ethylene acts upon the auxin in the plant, thereby affecting growth. As previously mentioned, this is apparently true in the case of geotropism. In view of the experiments described here, however, it is certain that other effects cannot be

completely explained in this manner, although it is possible that ethylene significantly influences auxin destruction in intact pea plants. Consequently it is very unlikely that ethylene affects directly the action of auxin on stem elongation.

The possibility that ethylene acts directly on the plant, even though it acts differently from auxin, will now be considered. From its effect on intact plants, it might be assumed that ethylene acts directly to decrease elongation without directly affecting auxin or other growth factors. But if ethylene acts directly to inhibit elongation in the intact plant, it should do likewise in the *Avena* test and the pea test. Experiments, however, show that these tests are affected only by pretreatment and that neither of them is affected by ethylene treatment during the test (tables 2 and 6). In view of this it is improbable that ethylene acts directly on elongation.

The experiments with oat and pea seedlings indicate that ethylene causes an activation or accumulation of food factor or some other factor promoting growth. This may be a direct action of ethylene on the growth factor in question, but in this case it is not clear why ethylene should decrease growth of the intact plant. It is also possible that ethylene acts in some other way to decrease growth and that this growth factor accumulates as a result of the decreased growth. This, however, fails to agree with the experiment in which pea stems were treated with ethylene only on one side, for these stems either did not grow or grew equally on both sides, yet the ethylene-treated side was more sensitive to auxin.

Action of ethylene in formation of stem swellings.—From the experiments described here it is impossible to say what mechanism is active in the formation of these swellings, but a few conclusions can be reached as to the probable relationship between ethylene and auxin in the formation of swellings. First, as was previously indicated, auxin in low concentrations is a prerequisite for the formation of swellings; for ethylene does not induce swelling formation when the supply of auxin is completely removed. Here auxin probably has the same rôle as it does in elongation of the oat coleoptile—namely, to increase plasticity of the cell wall, permitting the cell to change in shape and size.

Secondly, auxin in abnormally high concentration has in common with ethylene the ability to cause swelling of the stem. For the formation of these swellings, it is also necessary that some reaction preparatory to this change must occur in the roots of the plant; for if the roots are removed for as long as two days before the auxin or ethylene treatment, the stem fails to enlarge. Possibly this preparatory reaction involves the production of a substance or substances in the roots which are necessary for the formation of swellings, but it may also be some other type of reaction.

Here, as in the case of stem elongation, the action of ethylene is of a complex nature and probably indirect, for both auxin and some substance or reaction depending upon the roots are necessary before en-

largement occurs. It has also been shown (Michener, 1935) that ethylene does not act directly to cause root formation.

Comparison of the action of ethylene with that of auxin.—The similarities between the physiological effects of ethylene and those of auxin have been pointed out by Crocker, Hitchcock, and Zimmerman (1935). From the experiments described here, however, it is clear that the activity of ethylene differs widely from that of auxin and that there is no general similarity between the two. It has also been shown (with the pea test and with the formation of stem swellings on peas) that ethylene is completely unable to stimulate growth when no auxin is present.

The ability of ethylene to cause enlargement of stems is the only case considered here in which ethylene and auxin appear to act in the same way, and even here auxin plays two rôles. In the first, increase in cell wall plasticity, it is essential, and without it no swelling can occur. In the second, that of causing an unknown reaction necessary for swelling, it can be replaced by ethylene.

Is ethylene a hormone?—As previously mentioned, it has been suggested by Crocker, Hitchcock and Zimmerman (1935), that ethylene is to be considered as a hormone, since it is produced within the plant and also has physiological effects upon the plant. Proof is lacking, however, that ethylene ever affects tissues in which it is produced, except in ripening fruits and under experimental conditions in which a large amount of tissue is confined in a small space, preventing the escape of the ethylene produced. The known effects of ethylene are caused by a much higher ethylene concentration than can conceivably exist in the tissue of a plant growing in the open air, for ethylene is certainly produced only in very small quantities.* As the concentration of externally applied ethylene is decreased, its effects disappear and no new ones appear which are not produced by ethylene in the concentrations used in the experiments described here. When no ethylene is applied to the plant from the outside, there remains that which is produced within the plant, but how are we to know whether this is physiologically active or whether it is, in this concentration, merely an inert by-product of metabolism? It seems impossible to answer this question from data now available, for we have no way of knowing whether the plant would be affected by complete removal of the ethylene which it produces.

SUMMARY

Ethylene decreases longitudinal growth in intact oat and pea seedlings. It does not, however, influence production or transport of auxin. In the pea seedlings it is possible that it significantly influences destruction of auxin in the intact plant.

Ethylene also increases the sensitivity of these plants to auxin. It probably does so by causing an accumulation or activation of the "food factor" described by Went.

*According to Gane (1934), an apple may give off only 1 cc. of ethylene during its life.

Ethylene does not resemble auxin in its action on stem elongation.

It is difficult to explain the known facts on the basis of a direct effect of ethylene on elongation.

The manner in which ethylene decreases stem elongation thus still remains in doubt, but it is not due to any effect of ethylene directly on auxin or on the action of auxin on the plant. Also it is probably not due to a direct effect of ethylene on elongation.

Ethylene produces stem enlargements in pea and oat seedlings which closely resemble those produced by auxin in high concentration. The formation of these swellings depends on the presence of roots.

Auxin in high concentration and ethylene appear to act in the same way in the formation of these stem enlargements; but ethylene cannot induce swelling formation unless auxin is present in low concentration.

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THE FORMATION OF NODAL ADVENTITIOUS ROOTS IN *SALIX CORDATA*¹

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It is well known that stem-cuttings of certain willows, poplars, and other plants root very readily when placed under favorable conditions and that the adventitious roots usually appear at definite places on the stems; but no detailed investigation of the time and method of development of such roots has been published.

Trécul (1846) studied the position of adventitious roots on cuttings of many species and suggested the possibility of the existence in certain species of primordia of roots in the stems which remain latent until the cuttings of these plants are placed under conditions favorable for the growth of roots. Vöchting (1878) noted the regular arrangement of roots on cuttings of willow and other plants and found the primordia of roots in twigs three to four months old. He thought that the primordia must be present in still younger twigs, but too small to be seen. Van der Lek (1924) made a more detailed study of root-formation in cuttings of several species of *Salix* and *Populus*, in *Ribes nigrum*, and in *Vitis vinifera* and found root primordia in cuttings made in January, February, and March. In *Ribes nigrum*, primordia occur in three double series beginning immediately under the insertion of each leaf and extending downward through the internode; a few appear in one or more vertical series on the opposite side of the internode. The primordia lie outside the cambium. Those in series directly beneath the leaf are formed in those rays limiting the wood sectors which become the traces of the leaf in question; those on the opposite side are similarly related to the leaf next above. The rays are locally widened at the places where the root primordia appear. Each primordium is formed immediately under a lenticel. In *Salix*, Van der Lek found both nodal and internodal root primordia, the nodal ones "closely connected with the wide radial bands of parenchyma formed by the leaf strands leaving the wood cylinder." The internodal ones are similar in location to those of *Ribes nigrum*. In *Populus*, the root primordia are formed opposite the centers of the sectors which become the traces to the leaf above, rather than opposite the rays which bound these sectors. No pre-formed root primordia were found in *Salix caprea*, *S. aurita*, *Populus alba*, or *Vitis vinifera*.

The present study was made to determine the time of origin and the method of development of the latent root primordia in nodes of stems of *Salix cordata* Muhl. Beginning early in May, when the buds had just begun to grow, shoots of this willow were collected at intervals until early autumn. Nodal regions of shoots were prepared for sectioning in the

usual manner. All except the youngest shoots were separated into upper, median, and lower nodes. At intervals, beginning in January, cuttings of twigs from the previous season's growth were placed in a jar of water, through which air was bubbled, and each day, until the roots had emerged, nodal pieces of these cuttings were collected for a study of the resumption of growth of the root primordia.

With the exception noted below, a maximum of five adventitious roots may appear at each node of a cutting. The first two to emerge appear just below the stipule scars, the third directly above the insertion of the bud, and two later ones appear below the bud, on either side of a median line passing vertically through the bud (fig. 1). If two roots are formed directly below the bud, they are usually at different levels. Sometimes, but infrequently, an additional root appears later, very closely associated, either above or below, with any of the earlier ones. In addition, a few adventitious roots appear much later, scattered here and there along the internodes. The lateral nodal roots—those appearing just below the stipule scars—are usually larger than the others. They are more regularly present also, having been found on 150 of 200 nodes selected at random for examination. A root was found above the insertion of the bud in about half the cases, and one or two below the bud on only 40 of the 200 nodes examined. The appearance of two roots below the bud is a rare occurrence. No roots are present on several (usually three or four) of the lowermost nodes of the new shoot—that is, nodes just above the bud-scale scar. At these nodes only rudimentary or small dormant buds are produced.

A study of the internal structure of the stem at a node shows that three widely separated vascular bundles leave the vascular cylinder, extend as leaf traces through the cortex, enter the petiole separately,

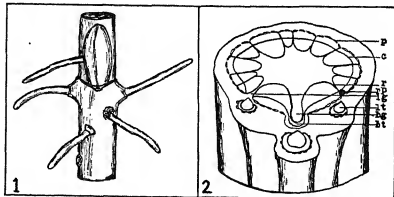
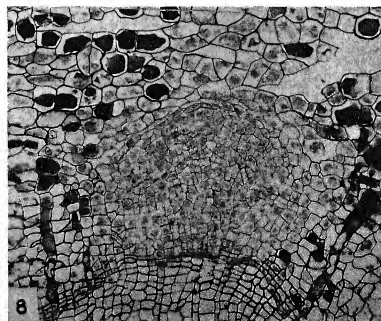
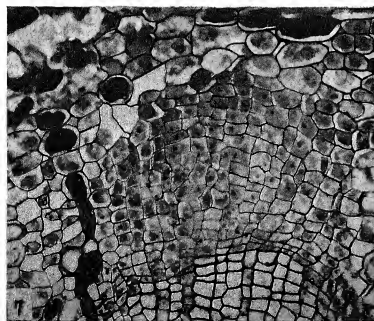
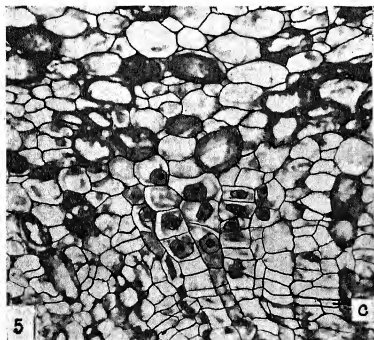
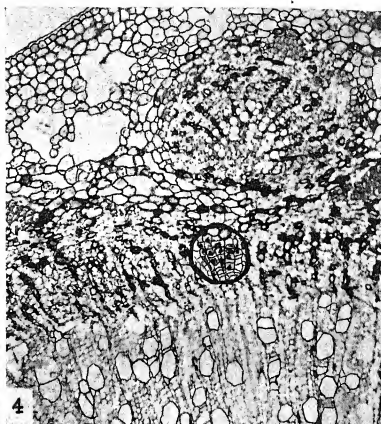


Fig. 1-2.—Fig. 1. Nodal region of stem-cutting; five adventitious roots, any or all of which may develop; the lower right root emerging through a lenticel.—Fig. 2. Node, with cross section at level of a primordium of an adventitious root; *bt*, branch trace; *lt*, leaf trace; *bg*, branch gap; *lg*, leaf gap; *rp*, root primordium; *r*, ray; *p*, pericycle; *c*, cambium; *pph*, primary phloem; *sph*, secondary phloem. (Same legend in following figures.)

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I wish to express my thanks to Misses Eleonora Kryski and Claire Nemecek for assistance in the preparation of materials for this study.



and join to make one bundle passing through the center of the petiole. The two lateral leaf traces begin at a higher level than the median one, extend vertically a short distance, then pass obliquely through the cortex to the leaf base. Sometimes one lateral trace begins at a slightly lower level than the other. The gaps in the vascular cylinder opposite the lateral leaf traces are short; the gap opposite the median trace is longer and broader and is continuous with the branch gap above. The gaps taper at each end and merge with rays above and below. A diagram of a portion of a node (fig. 2) shows the three leaf traces in the cortex: the median one, in the midrib region of the leaf base; the one on the left, entirely separated from the vascular cylinder and extended toward the median trace; the one on the right, extended still more than the left one toward the median trace. The left leaf gap is present in the vascular cylinder, but the right gap is below the plane of the section. A vascular ray which extends upward from the right gap is seen. The branch traces and the branch gap are found at the level of this section, but the median leaf gap is below and does not show at this level. Figure 3, a portion of a transverse section of a lower node of a young branch (collected May 26), shows a lateral leaf trace extended in the cortex upward and slightly toward the right from the gap where it emerged from the vascular cylinder. The region opposite the pointed projection of the pith is just above the leaf gap. It contains secondary phloem and xylem, and cambium, but no primary phloem and xylem. Of the several rays present, one is continued upward from the leaf gap (primary), and the others are produced by the cambium (secondary). The pericycle, which is sclerenchymatous on the outer side of the primary phloem, seems to be interrupted opposite the region above the gap, and parenchyma fills the space between the secondary phloem and the trace. Large air spaces are present in the cortex to the left of the leaf trace. A few cells in the outer pith and cortex, and many in the rays, especially in the phloem rays, are filled, or partly filled, with tannin. These appear black in the photographs. No endodermis nor starch sheath can be distinguished, but the cells immediately outside the pericycle form a fairly regular layer bounding the stele.

Since the lateral nodal roots occur at each node so much more frequently and regularly than do the others, they were studied more extensively. The primordia of these roots are conspicuous and well formed at the end of the growing season. They are above the lateral leaf gaps and associated with the ray

which continues upward from the gap—on that side of the ray away from the leaf base. At the level of a primordium, the leaf trace lies in the cortex, definitely separated from the vascular cylinder and advanced slightly toward the base of the leaf. The primordia are outside the cambium, with secondary phloem adjacent laterally, and cortical parenchyma toward the outside (fig. 2).

The first evidence of the formation of a root primordium is the absence of starch, tannin, or other stainable reserve materials from the region above a leaf gap (fig. 3). The tissue outside the cambium in this region does not differentiate into sieve tubes and companion cells, but becomes parenchymatous. The cells, which are arranged in very regular rows, are of approximately uniform size and are usually rectangular in section. The ray cells are so similar to the other cells that it is difficult to distinguish them. In sections of living stems this tissue appears much more transparent and colorless than the adjacent secondary phloem. There seems to be no name other than secondary parenchyma to apply to this tissue. It originates from the cambium and occupies the place of the secondary phloem, but it has none of the characteristics of the typical secondary phloem which lies on either side.

The cells in the outer part of a ray in this secondary parenchyma now enlarge, their cytoplasm becomes denser so that they stain more deeply, and the nuclei increase in size. The extraordinary size of the nucleoli is especially noticeable. Cells on either side of the ray, but chiefly on the side away from the median leaf trace, undergo similar changes until a group of cells, distinctly different from the neighboring ones, is discernible. Such a group, including in section about 12 or 14 cells, is seen inside the circle in figure 4. The relation of this group to the leaf trace and to the tissues of the vascular cylinder is evident. It lies without the cambium, and within the parenchyma which occupies the space in the interrupted cylinders of pericycle and primary phloem. This group of enlarged cells, arranged as a wedge with its broad side toward the cortex, constitutes a primordium of an adventitious root. An enlargement of the primordium (fig. 5) shows the changes in cells and nuclei already mentioned, as compared with cells of the secondary phloem on either side or of the cortical parenchyma toward the outside.

The root primordium continues to increase in size as neighboring cells undergo changes similar to those just described (fig. 6). Soon secondary cells outside the cambium, including several rays and all the cells between them, are involved. Division of the enlarged

Fig. 3-8.—Fig. 3. Portion of lower node from new branch (May 26), in cross section; lateral leaf trace; large air spaces in cortex on left of trace; region in vascular cylinder above leaf gap, where primordium is destined to be; no primary xylem or phloem present in this region; pericycle interrupted across this region.—Fig. 4. Cross section of branch taken June 10; early stage in formation of primordium (within circle).—Fig. 5. Enlargement of above primordium; outer part of vascular ray and adjacent cells involved.—Fig. 6. Older primordium from branch removed June 26; cells of several rays and those between have become meristematic; typical secondary phloem on either side of primordium; dome-shaped protuberance on secondary xylem.—Fig. 7. Still older primordium; dividing meristematic cells in fairly regular rows; no differentiation into histogens of root.—Fig. 8. Primordium from cutting of one-season-old stem in water Jan. 27-30; growth of primordium resumed.

cells takes place, chiefly in the tangential, but also in the radial and transverse planes. The daughter cells do not enlarge between divisions to the size of the mother cells, and therefore the primordium comes to consist of a large number of small meristematic cells occupying the same space as that occupied by the mother cells before active divisions began. The cambium adds cells to the inner side of the primordium. Typical secondary phloem can be seen on either side of the primordium in figure 6. The vascular (secondary) rays can be followed through the secondary xylem and phloem, except in the region of the primordium, where the rays appearing in the xylem seem to end at the cambium. Many of the cells of the phloem rays are filled with tannin, as seen to the right of the primordium in figure 6. A lateral leaf trace which lies beyond the tannin-filled cells in the upper right corner, but does not show in the figure, serves to locate the primordium with respect to the stem structure.

A still older root primordium is shown in figure 7. It has increased in size tangentially by the incorporation of adjacent secondary phloem cells and radially by the activity of the cambium. The small meristematic cells of the primordium are still arranged in radial rows, but irregular divisions begin to take place in the central part. The cells, appearing somewhat plasmolyzed in the figure, possess dense cytoplasm and the characteristically large nuclei and nucleoli.

While a primordium is forming, the cells of the adjacent secondary xylem enlarge, producing a protuberance which pushes the center of the primordium outward and makes it somewhat dome-shaped (fig. 6). If the bark were removed, this protuberance would appear as a hemispherical hump on the wood cylinder. The cells grow both radially and tangentially. Only the outer several layers of cells are involved in this enlargement.

No primordia were found in any of the nodes sectioned from the collections of May 2, 11, or 26. Lower nodes, but not the median or upper nodes, of stems collected June 10 and 26 contained early stages of primordia. In the collections of July 30 and August 16, older primordia were found in the lower nodes, younger ones in the median nodes, and none in the upper ones. In the September 27th collection, they were found in lower, median, and upper nodes, but not in the very young nodes at the tips of the stems. No primordia were ever present in the nodes which bear rudimentary buds at the bases of the branches. From these facts it can be concluded that the primordia of roots begin to develop in early June in those lowest nodes of the new shoots which bear well-differentiated buds and that they appear in the other nodes progressively upward throughout the growing season. By late autumn, fully formed primordia are present in most nodes, except those at the extreme tips, of all new branches. These primordia remain dormant throughout the winter and perhaps for many years.

The time of the appearance of the first indications of primordia seems to be correlated with the age and stage of differentiation of the tissues of the stem and must, therefore, vary with the climatic conditions which affect rate of growth and differentiation. They begin to form when a considerable amount of secondary tissues has been produced, approximately at the stage shown in figure 4. At this time all the primary tissues except the collenchyma and the pericycle sclerenchyma are mature.

The primordia of the roots which sometimes emerge from behind the buds originate and develop in the same manner as those already described. Such a primordium originates in a ray, sometimes immediately above a branch gap, but sometimes farther up the stem. It is interesting to note that a protuberance on the xylem is also associated with such a primordium. An early stage in the formation of one of these primordia was found in a lower node from stems collected June 10, showing that they may be produced at the same time as those of the lateral roots. The evidence is insufficient to determine whether or not the primordia above the insertions of the buds are produced in successively higher nodes as the season advances, but this may well be the case.

Sections of many stems cut below the attachments of the leaves were examined, but no primordia of roots were found in these regions. This fact is not surprising, since roots arising in this position on cuttings are comparatively rare. It will be interesting to study the primordia of such roots, since they are not associated with the unique anatomy of nodes but with ordinary internodal structure.

In case a root does not appear at one of the customary nodal positions, a primordium has failed to develop at that point. Secondary phloem then differentiates normally above the gap, and no protuberance is formed on the xylem cylinder.

By the end of a growing season, the primordia have developed in the newest branches to the stage seen in figure 7. If the branches are left on the tree, the primordia remain dormant. If detached stems are placed in water or moist sand, the primordia resume growth. Figure 8 shows a primordium of a lateral root in a cross section of a cutting removed from the tree on January 27 and placed in water for three days. It has elongated radially, enlarged tangentially, and become more like a root tip. The cells on the outer edge are stretched and flattened because the primordium is beginning to push into the cortex. The root tip continues to grow outward, the histogens appear, and soon the primary tissues differentiate in the manner typical of adventitious roots. The elongating root pushes through the cortex, collenchyma, and epidermis. It may be recalled that opposite a primordium the primary phloem and pericycle are absent and that the cortex is loosely arranged, with large intercellular spaces, and, therefore, there is little resistance to the passage of the growing root. The root emerges through a lenticel only if the latter happens to be opposite the definite place where a

primordium is formed. The lower right root in figure 1 has emerged through a lenticel.

Although Van der Lek reported that adventitious roots in cuttings of such plants as willows and poplars are associated with rays and cambium, the exact rôle of these tissues in root formation has not been previously known. The present study shows that primordia of nodal roots of *Salix cordata* originate in the outer part of rays, above leaf gaps, and that they are later enlarged by the addition of cells from the cambium. Little is known of the causes underlying the formation of root primordia. Most attempts to investigate these causes have been made with cuttings, in which primordia originate after wounding and removal of a part of a branch from the rest of the plant, with undoubted resulting changes in the behavior of the part removed. These factors are not involved when plants produce primordia in the growing branches. Since in *Salix cordata*, the primordia of adventitious roots originate in definitely predictable locations, and since they may appear on one side of a node and not on the other, or at one node and not at the node above or below, it seems possible that differences in behavior between primordium-forming and primordium-lacking regions might be detected by microchemical investigation. Differences might give a clue to causes of the origin of root primordia. Such a study is planned for the future.

SUMMARY

Roots arise on stem-cuttings of *Salix cordata* from definite places at and near the nodes. The nodal roots develop from primordia which are present in the stems before their removal from the tree. A pri-

mordium is formed from parenchymatous secondary cells, above a leaf or branch gap. The cells in the outer part of a vascular ray in this region enlarge and become meristematic. Neighboring cells undergo similar changes until the secondary cells outside the cambium, including several rays, are involved in the formation of the primordium. The cambium adds cells to the inner surface of the primordium. A hemispherical protuberance, formed by the enlargement of cells of secondary xylem adjacent to the primordium, pushes the center of the primordium outward, making it dome-shaped.

The primordia appear in early June in the lower, but not in the three or four lowermost, nodes of the developing branches. They are formed progressively upward in the nodes of the growing branches during the summer. By autumn they have reached their maximum development except in the youngest nodes. They remain in a dormant condition unless the branches are removed from the tree and placed in conditions favoring the growth of the primordia into roots.

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A CHARACTER ANALYSIS OF COLONIES OF IRIS FULVA, IRIS HEXAGONA VAR. GIGANTICAERULEA AND NATURAL HYBRIDS¹

Herbert Parkes Riley

THE IRISES of southern Louisiana have been of peculiar botanical interest since they were first called to the attention of botanists by the late Dr. John K. Small a little more than a decade ago. Many of these irises differ to a greater or lesser extent in the size and shape of the flower parts and in the amount and distribution of pigments in the flower, and there is so much variation among them that Small and Alexander (1931) have listed more than eighty species from within the borders of Louisiana. This interpretation of the species, however, has not been accepted by all botanists, for Viosca (1935), chiefly on the basis of ecological evidence, recognizes but four species from the same territory and maintains that the other types which are listed by Small and Alexander are hybrids. In a more recent paper, Foster (1937) agrees essentially with Viosca. He considers *I. virginica* L., *I. fulva* Ker-Gaw., and *I. brevicaulis* Raf. (= *I. foliosa* Mackenzie and Bush) to be the only species in Louisiana, regards *I. giganticaerulea* Small as a variety of *I. hexagona* Walter, and believes with Viosca that many of the remaining types are the result of natural hybridization between *I. fulva* and *I. hexagona* var. *giganticaerulea* (Small) R. C. Foster. Throughout this present paper, Foster's treatment of the genus will be followed, since it is the only comprehensive treatment of the American species of *Iris*. Foster's study has been largely taxonomic but has combined chromosome counts with a study of morphological characters.

Cytological and ecological approaches are of great value in supplementing the methods of pure taxonomy and especially in determining the probable hybrid origin of a given type of plant. It is possible that biometric methods may in certain cases furnish even more precise estimates of hybridity, especially such methods as have been suggested by Anderson (1928, 1936). An attempt to apply these methods to some of the Louisiana irises is reported in this paper.

MATERIALS AND METHODS.—This investigation represents a comparative study of seven characters on plants of four populations, which consist of a group of clones² of *Iris hexagona* var. *giganticaerulea*, a group of clones of *I. fulva*, and two groups of clones located geographically between the other two groups. These four colonies of wild plants were found growing in a region between Thibodaux and Vacherie, which has often yielded a large number of *Iris* varieties. As the inhabitants of that area have been

repeatedly annoyed by parties of *Iris* collectors during recent years, and as they have come to believe that the plants growing on their land have commercial value, it was only through the influence of Mr. Viosca that the author was permitted to collect flower-stalks in that area. In this general region, two main types are found in great abundance. *I. hexagona* var. *giganticaerulea* grows in the low lands in rich, mucky clay with a high water content, while *I. fulva* is to be found on somewhat drier land in woods and on the slopes of alluvial ridges. The four groups of plants concerned in this study are listed as Colony G, which includes plants 1 to 23, Colony H1, including plants 101 to 123, Colony H2, made up of plants 201–223, and Colony F, which consists of plants 301 to 323.

Colony G consisted of twenty-three clones which proved to be *Iris hexagona* var. *giganticaerulea*. These clones were collected at random in an area which contained a much larger number of clones, all of which showed little variation from one another in external appearance, and which were growing together in marshy land along the side of Bayou Boeuf (fig. 1). Colony G did not show any appreciable variation, but Colony H1 exhibited much. Since the latter was the first to be studied and consisted of twenty-three clones, populations of similar size were selected for Colonies G, H2, and F, as populations of like size are more readily compared.

Colonies H1 and H2 were located near one another in a pasture about 500 feet from Colony G in land that was formerly a deltaic stream. On both sides of this stream was the alluvial ridge. As is seen from the map in fig. 1, Bayou Boeuf, which is a swamp drainage bayou connecting Lake Boeuf and Lake des Allemands, has cut across this former deltaic stream and its alluvial ridges. The ridges are wooded except where the edges adjoining the former stream have been cleared for houses and for a road. Heavy vegetation in the old stream bed has been somewhat kept down for the past dozen years by pasturing. Colony H2 was found at about the center of this former deltaic stream, but Colony H1 was to one side, nearer one of the ridges. Colony H1 contained twenty-three clones, some of which were obviously different from those of Colony G and appeared upon casual inspection to exhibit some characters shown by both *Iris hexagona* var. *giganticaerulea* and *I. fulva*. Colony H2 also showed some apparent hybridization but to a much lesser extent. It was about fifteen to thirty feet from the clones of Colony H1 and nearer the center of the bed of the abandoned stream. Perhaps all the plants in this pasture should have been treated as one colony, but those of Colony H1 were separated

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² A clone is a variety propagated asexually from a single original individual.

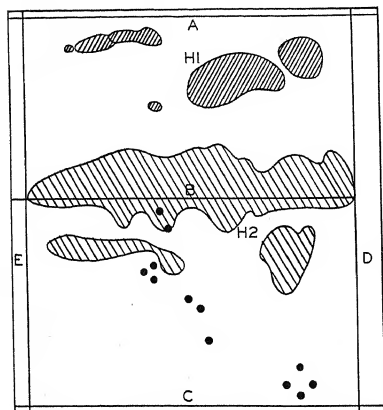
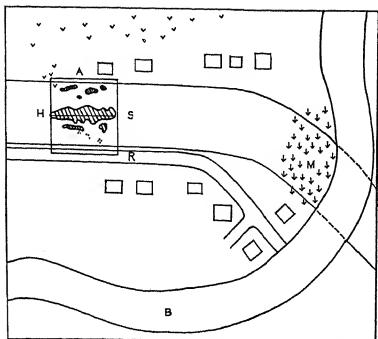


Fig. 1. A diagrammatic map of the general region showing the abandoned deltaic stream (S) traversed by Bayou Boeuf (B); at the intersection is a marsh (M) in which Colony G was growing. The plants of var. *gigantenerulea* are indicated by upright arrows. The edge of the alluvial ridge is at A, and R represents the public road. *I. fulva* is indicated by v's, and in the rectangle (H) are found Colonies H1 and H2. Colony F is not included in the figure.

Fig. 2. An enlarged view of H of fig. 1. The area is bounded by the alluvial ridge (A), the edge of the road (C), a footpath (D) and a wire fence (E) and is bisected by another wire fence (B). Oblique lines from the upper right to lower left indicate Colony H1, while Colony H2 is included in the area cross-hatched from upper left to lower right. Black dots indicate cypress trees.

from the others by a strip of land free from *Iris* and fifteen feet in width at the narrowest parts (fig. 2). This division into two colonies was a convenient one for purposes of study and, as it has turned out, was a logical one, as the composition of the two colonies proved to be somewhat different, as will be shown below. Colony H2 was growing near a fence which separated the pasture into two fields and which was standing in the center of the former deltaic stream. The land occupying the bed of this former stream was from several inches to a foot or two lower than that of the alluvial ridges. On the Mississippi delta, a difference of a few inches in elevation may have a much greater effect ecologically than several feet in many other parts of the world (Viosca, 1933).

All four colonies were studied in April, 1936. One stalk from each of the twenty-three clones of colonies G, H1, and H2 were cut off at the ground, and measurements and other records, except pollen fertility, were taken in the field upon all the stalks of one colony at a time. Anthers of open flowers on each stalk were placed in vials of acetic-alcohol in which they were kept until it was convenient to determine the percentages of fertile pollen. The author was very fortunate in having Dr. Edgar Anderson and his assistant, Mr. Leslie Hubricht, with him to aid in the scoring in the field.

Colony F consisted of clones of *Iris fulva* Ker-Gawler located in an oak forest on the same alluvial ridge that adjoined colonies H1 and H2, but a few miles from the location of these other colonies. One stalk of each of twenty-three clones was clipped off at the ground as in the case of the other colonies, but, as circumstances made it impractical to score these plants in the field, the stalks were placed in water and brought to New Orleans, where the analysis of the characters was made later that night.

The method of analysis is that described by Anderson (1936) and consists in selecting a number of characters with respect to which two species differ and recording each character of each plant as identical with one of the species or as intermediate between them. Each character is then assigned an arbitrary index value. When each character on a plant has received a value on this basis, the total index value of that plant is obtained by summing the values of all its characters. When an index value has been assigned to all the plants or clones of a population, a frequency distribution can be made of the population as a whole; a comparison of the frequency distributions of two such populations will give a general picture of the two populations.

The characters chosen for this type of study must depend upon the genus investigated. They need not be characters which can be expressed in terms of numbers, grams, or centimeters; in fact, the value of this method lies in its ability to deal with purely qualitative characters and treat them quantitatively. In general, the selected characters should be: (a) readily observed macroscopically or at least with the hand lens; microscopic characters may be used but

add considerably to the labor involved; (b) discontinuous (not overlapping) in the two species, if possible; (c) easily divisible into a few simple categories, if there are intermediates; (d) the result of the interaction of several genes, although they may be due to a single pair of factors; in this latter case if one gene exhibits dominance, the hybrids will be like either parent, and there will be no intermediates.

The method of selecting the characters and of assigning numerical values to them is purely arbitrary. No implication that two characters are equally important should be drawn from the fact that they have equal index values, nor should a character necessarily be considered as more important than another because it has a larger value. The index value of a character is usually determined by the number of intermediates which can be clearly identified between

mined plant between the two types it was a simple matter to compare it with them and score it. The numerical values assigned were 2 to plants with pure green tubes (like var. *giganticaerulea*), 0 to those with as much golden pigment as *I. fulva*, and 1 to all intermediates regardless of the depth of the yellow color.

Color of the blade of the sepal.—In scoring this character, the Fischer Color Chart published by the New England Gladiolus Society was used. This chart does not give the fine distinctions of color and shade that are found in Ridgeway, Maerz and Paul and some other color charts; for that reason it is more satisfactory for a rough analysis of this sort, since the scoring can be done more quickly. In this chart, the most important colors are spread out like the spokes of a wheel, and a rough but adequate color

TABLE 1. Analysis of the characters of each plant of Colony G with their index values and pollen fertility.

Plant number	Tube color	Color of sepal blade	Sepal length	Petal shape	Stamens	Style appendages	Crest	Index value	Pollen fertility
1	g	VB5	8.8	g	g	g	g	17	94.6%
2	g	VB4	8.5	g	g	g	g	16	93.7
3	g	VB4	9.4	g	g	g	g	17	97.0
4	g	BV4	9.4	g	g	g	g	17	94.9
5	g	BV5	10.0	g	g	g	g	17	94.1
6	g	BV5	9.1	g	g	g	g	17	95.9
7	g	VB5	10.8	g	g	g	g	17	92.1
8	g	V2	8.9	g	g	g	g	16	91.6
9	g	BV4	9.3	g	g	g	g	17	89.0
10	g	BV3	9.0	g	g	g	g	17	98.3
11	g	BV4	9.0	g	g	g	g	17	93.1
12	g	VB6	11.0	g	g	g	g	17	
13	g	BV5	9.7	g	g	g	g	17	93.8
14	g	VB4	8.8	g	g	g	g	17	94.0
15	g	BV4	10.1	g	g	g	g	17	84.3
16	g	VB5	9.5	g	g	g	g	17	90.0
17	g	VB3	9.0	g	g	g	g	17	
18	g	VB5	9.7	g	g	g	g	17	93.2
19	g	VB3	9.2	g	g	g	g	17	
20	g	VB5	9.6	g	g	g	g	17	92.3
21	g	BV5	10.1	g	g	g	g	17	91.7
22	g	VB6	9.9	g	g	g	g	17	92.2
23	g	VB5	9.5	g	g	g	g	17	

the two species. This tends to lend too great weight to some characters, but until the method can be refined so as to evaluate characters more accurately, the present arbitrary method will serve as a first approximation.

In this *Iris* investigation, seven characters were studied. They are listed below with the index values assigned to each.

Tube color.—In *I. hexagona* var. *giganticaerulea* the tube or hypanthium is green, while in *I. fulva* it is golden-yellow. A flower of each of the two species was placed on a notebook with a space between for the unknown. By placing the flower of an undeter-

determination can be made very rapidly. The symbols V.B., B.V., etc., of the chart are used in this paper in tables 1 through 4 but are condensed to VB, BV, etc. Intensity of color is expressed by assigning the number 1 to the deepest shade of any one color on the chart and number 6 to the palest, so that VB1 in the tables indicates a deep violet-blue, while VB6 indicates a very pale violet-blue. The range of pure var. *giganticaerulea* lies within the blue-violets and violet-blues, and the index value 4 was assigned to plants falling within these categories. On the other hand, the coppery red of *I. fulva* resembled most nearly the reds or orange-reds of the Fischer

chart, and these colors were assigned the value 0. Between the blue-violet and red are three colors. To violet was assigned the value 3, while red-violet was numbered 2 and violet-red 1. Thus the intermediate colors are classified into three grades. Index values are based on the color alone, and depth of hue is disregarded, as it seems to have little significance, if any. This is a purely arbitrary method and, perhaps, gives too great weight to this character in determining the total index value of a plant. However, it must be remembered that the color of the blade of the sepals has been regarded as an important taxonomic character in these species by both Small and Viosca.

types was as follows: measurements of sepals on 110 plants of this species from this and other localities had been obtained in connection with another type of study which has not yet been published. These plants had not been measured for this present study and were undoubtedly as uncontaminated specimens of *I. fulva* as one can obtain in Louisiana; therefore, they were well suited to serve as impartial standards of *I. fulva* for this investigation. The mean length of sepal of these 110 plants was 5.861, and the standard deviation was 0.513. The upper boundary of pure *I. fulva* was regarded as the mean plus the standard deviation or 6.374 cm. All plants which had sepals which were 6.4 cm. or less in length were

TABLE 2. Analysis of the characters of each plant of Colony F with their index values and pollen fertility.

Plant number	Tube color	Color of sepal blade	Sepal length	Petal shape	Stamens	Style appendages	Crest	Index value	Pollen fertility
301.....	f	R3	5.4	f	f	f	f	0	98.1%
302.....	f	R3	6.1	f	f	f	f	0	97.1
303.....	f	R3	5.5	f	f	f	f	0	99.3
304.....	f	R3	5.9	f	f	f	f	0	94.9
305.....	f	R5	6.6	f	f	f	f	1	95.3
306.....	f	R4	5.8	f	f	f	f	0	98.7
307.....	f	R5	6.0	f	f	f	f	0	98.2
308.....	f	R3	5.9	f	f	f	f	0	94.5
309.....	f	R3	5.9	f	f	f	f	0	98.9
310.....	f	R3	5.5	f	f	f	f	0	97.1
311.....	f	R3	5.6	f	f	f	f	0	93.2
312.....	f	R3	6.8	f	f	f	f	1	99.4
313.....	f	R3	6.4	f	f	f	f	0	96.6
314.....	f	R3	6.0	f	f	f	f	0	98.2
315.....	f	R3	6.0	f	f	f	f	0	97.1
316.....	f	R3	6.0	f	f	f	f	0	98.9
317.....	f	R3	5.1	f	f	f	f	0	97.5
318.....	f	R5	6.0	f	f	f	f	0	89.5
319.....	f	R3	5.5	f	f	f	f	0	93.1
320.....	f	R4	5.7	f	f	f	f	1	98.3
321.....	f	R5	5.3	f	f	f	f	0	96.1
322.....	f	R3	6.1	f	f	f	f	0	98.2
323.....	f	OR4	6.6	f	f	f	f	1	98.1

Sepal length in centimeters.—This character was more difficult to score than any other, although the measurements themselves were easy to obtain. The length was measured from the rounded tip of the blade to the point where the end of the claw joins the tube. The latter point was determined by breaking the sepal off from the tube and considering the place it broke off most easily as the place where it was naturally joined. The range in sepal lengths extended from 5.1 in a typical *I. fulva* to 11.0 in a plant was unmistakably *I. hexagona* var. *giganticaerulea*. To decide, however, what should be considered the limits of both species and into how many categories the intermediates should be divided was not easy. The method finally adopted to determine the boundary between *I. fulva* and intermediate

regarded as pure *I. fulva* and given the index value 0. The same method was used for *I. hexagona* var. *giganticaerulea*. Sixty plants collected and measured for other purposes and from a very different locality were used as the standard. The mean sepal length of these plants was 9.525, and the standard deviation was 0.928. The line of demarcation between this variety and intermediates was considered to be 9.525–0.928, or 8.597; all plants with a length of 8.6 cm. or more were given the index value 3. Between 6.4 and 8.6, the highest value of *I. fulva* and the smallest value of var. *giganticaerulea*, there exists a number of values. These were divided up arbitrarily into two groups of intermediates, so that all plants with a length between 6.5 and 7.4 were given the value 1 and those between 7.5 and 8.5 were considered to have

an index value of 2. This whole distribution is arbitrary, but the author feels that the use of the mean and standard deviation to express the usual limits of the two species is as accurate as many expressions of length in taxonomic treatises. The values given to sepal length in *I. fulva* by Small and Alexander (1931) are 5-8 cm., by Viosca (1935) 2 to 3 inches (which brings them to about 7.6 cm.), and by Foster (1937) 5.5 cm. There is great diversity in the lengths expressed in these three papers, and perhaps the author is complicating the situation by taking 6.4 as the arbitrary upper limit. However, the upper limits of both Viosca and Small appear to be excessively high, as 7.0 was the largest of any of the 110 plants measured by the author and used as a basis in this paper. For *I. hexagona* var. *giganticaerulea*, Small and Alexander give 8.5-11.5 cm. as sepal length, while Viosca gives 3.5 to 4.5 inches (11.5 cm.), and Foster gives 8.6 cm. This latter figure is the same as that used by the author, but it must be remembered that it represents the lower limit of the species as interpreted in this paper and that the mean length is about 9.5 cm.

Petal shape.—The petals of var. *giganticaerulea* are cuculate-spatulate, while those of *I. fulva* are narrowly obovate with practically no claw. This character is not a measurable one and is purely qualitative. Plants which more nearly resemble var. *giganticaerulea* were scored as 2, those like *I. fulva* as 0, and those which seemed to be intermediates to a more or less degree between these two types, as 1. Typical examples of both species were collected, and all plants to be identified were referred to them in the same manner as explained for tube color.

Exsertion of anthers.—In *I. fulva* the stamens are slightly longer than the limbs of the styles, so that the anthers are exserted and protrude about a millimeter beyond the ends of the stylar limbs. In var. *giganticaerulea*, on the other hand, while both stylar limbs and stamens are longer than they are in the other species, the limbs of the style are proportionately much longer, and the distal ends of the anthers are consequently approximately a centimeter from the ends of the limbs of the style; these anthers are included. Intermediates may be found in which the proportionate length of the stamens is greater than that in var. *giganticaerulea* but less than that of typical *I. fulva*. Plants which resemble *I. giganticaerulea* are scored 2; those with exserted anthers are given the value 0, while intermediates are 1.

Appendages of the style branches.—In *I. fulva* these structures are small and just barely toothed, while in *I. hexagona* var. *giganticaerulea* they are much larger and deeply lacerate-toothed. Intermediate stages are found in which the size of the appendages and the depth of the indentations on their edges vary from one extreme to the other. Within the pure species, the variation is small, and by no possible chance could the stylar appendages of one species be mistaken for those of the other. Again, *giganticaerulea*

types are given the value 2, intermediates are 1, and plants resembling *I. fulva* are scored 0.

Crest.—This term is used in the sense in which it was used by Small and Alexander (1931). In *I. fulva*, the crest is absent or present only as a midrib of the claw, while in *I. giganticaerulea*, the crest is well developed and of several ridges; there is a large yellow crest-zone which is surrounded by a paler zone. The *fulva* type was scored as 0 and *giganticaerulea* as 2, and all intermediates, irrespective of the extent to which the crest had developed, as 1.

In studying these populations of *Iris*, every plant of each population was observed and classified as to each of these seven characters. Each plant, therefore, has seven numerical values, one for each character, and the sum of these seven numbers represents the index value of that plant. The highest possible index value which a plant may have according to the system of scoring used in this study is 17, and this number is characteristic of *I. hexagona* var. *giganticaerulea*; the lowest possible index value is 0 and represents a typical *I. fulva*. Between these figures are 16 possibilities, and the significance of these numbers in terms of species and hybrids will be discussed in the next section of this paper. The frequency distributions of the index values of all the plants of each of the four natural populations were plotted out as histograms, and the populations were compared in this manner.

With the exception of four plants from Colony G, pollen fertility determinations were made of all the plants of all five populations. Aceto-carmine mounts of anthers fixed in acetic-alcohol were used, and, when possible, more than 500 grains were counted; in very few cases were less than 200 grains used. The fertile grains swell and stain pink, while the sterile ones remain shrivelled and unstained and are apparently nothing more than empty spore walls. Pollen fertility was not used as a character to determine the indices of the plants but was used for comparison with the indices, to learn to what extent pollen fertility is a rough indication of hybridization in these species of *Iris*. The pollen fertility records of these plants have also been used in conjunction with another study in which it was shown that plants of *I. fulva*, *I. giganticaerulea*, *I. virginica*, and *I. brevicaulis* had a significantly higher percentage of fertile pollen than did those of certain of Small's "species" and that this could be used as evidence of the hybrid origin of the latter (Riley, unpublished data).

OBSERVATIONS.—*Colony G.*—Upon casual inspection, these plants all seemed to be *I. hexagona* var. *giganticaerulea*. The twenty-three clones selected for study were collected at random in a field containing perhaps a hundred, located in swampy ground by the side of a bayou. Twenty-one of the twenty-three clones scored the maximum number of 17 points. Plant 2 failed to score the maximum number, since the length of the sepal was a little less than that designated as typical *I. var. giganticaerulea* by this arbitrary method of scoring. Plant 8 was violet

in color and therefore lost one point on its total score. Only two of the plants of this colony scored less than 17 points and these only one point less. Intra-specific variation might well account for these two plants. The index values of all these plants are listed in table 1 and the distribution in figure 3.

Colony F.—Colony F was located in a region containing a large number of clones all of which appeared to be pure *I. fulva*, or as pure representatives of that species as are ever found in Louisiana. Twenty-three colonies were selected at random and were scored in the same manner as were those of Colony G. Of these, nineteen had a value of 0. The other four plants of this colony scored 1, which is not a significant deviation and is probably a normal fluctuation within this species. Plants 305, 312, and 323 deviated only in having slightly longer sepals than typical *I. fulva*, but in respect to sepal length this division into arbitrary categories may well be at fault. Plant 320 was typically *fulva* in every respect except that the crest was developed to a greater extent than usual, but this at least is a slightly varying character. Tabulations are found in table 2, and the distribution is expressed in the histogram in figure 3.

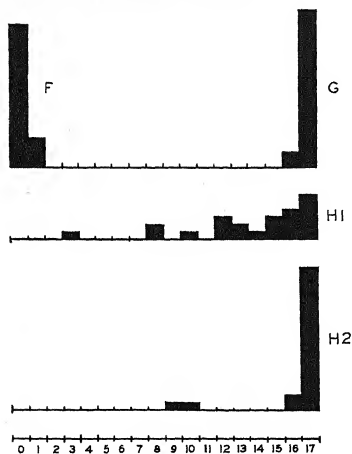


Fig. 3. Frequency distributions of total index values of Colonies G, F, H1, and H2.

Colony G is undoubtedly pure var. *giganticaerulea*, while Colony F is pure *I. fulva*. Pollen fertility records of these two species are interesting. In Colony G, over 90 per cent of the pollen was fertile in all the nineteen plants upon which records were taken except two; plant 9 had 11 per cent bad pollen, and in plant 15 the fertility was only 84.3 per cent. The lower fertility of the first of these two plants is not

significant; the second has an abnormally high sterility, but there is doubt as to the accuracy of the count of this plant. The vial which contained the anthers of plant 15 was lost, and the counts were made from anthers from an herbarium specimen which had been in the press for several days; this may well account for the fact that the fertility of this plant is lower than normal. In Colony F, all plants were very highly fertile except plant 318, in which only 89.5 per cent of the pollen was good. The percentage of fertile pollen on each of these plants is listed in tables 1 and 2.

Colony H1.—Colony H1 was located between Colonies G and F. A more explicit account of the geographical relations of all these colonies will be given in the discussion. Of the twenty-three clones of Colony H1, ten could be identified as *giganticaerulea* and had index values of 16 or 17. In each case the pollen fertility was 88 per cent or better. Three plants had values of 15; therefore, they deviated from the norm of var. *giganticaerulea* only slightly, but more than did any plant of Colony G. In the case of plant 112 the deviation was only in the length of the sepals which were considerably shorter than in plants of that variety. Plants 115 and 121 had sepals which were shorter than those of pure *I. var. giganticaerulea* but yet were longer than those of plant 112. Plants 115 and 121 were also intermediate in tube color. Pollen fertility was high for all these plants except 115, in which it was exceptionally low, being less than 50 per cent. Plants 108, 118, and 120 had index values still further from *I. hexogona* var. *giganticaerulea*. All were intermediate in tube color; 108 and 118 had violet colored sepals, but 120 was *giganticaerulea* in this respect; both 118 and 120 had sepals of intermediate lengths, but those of 118 were very short and almost approximated those of *I. fulva*; 108 and 120 were intermediate in the size and shape of the stylar appendages, and 120 was intermediate as to petal shape. Plant 108 scored 14, and plants 118 and 120 had index values of 13. Three plants had a total value of 12. Plants 110 and 113 were intermediate in tube color, sepal color, sepal length and crest, and had a low pollen fertility; plant 106 reached the same index value because it was intermediate in sepal length, tube color, petal shape, and stamen length and was also intermediate in sepal color, although it was nearer to var. *giganticaerulea* than plants 110 and 113, and therefore scored 3 points for this character instead of 2. These three plants illustrate that, taken as a whole, several plants may bear the same general relation to two species although they differ from one another in a wide variety of individual characters. Plant 106 had a higher percentage of fertile pollen than might be expected of a plant that shows intermediate characters; the pollen fertility of the other two plants was more normal, considering their index values.

Plant 109 was intermediate in tube color, petal shape, stamen length, and crest, and had short, red-violet sepals; these intermediate characters gave it

an index value of 10. The pollen fertility of this plant was only 69 per cent. Plants 101 and 105 had a value of 8 on this system and for very different reasons. Plant 101 was typical of var. *giganticaerulea* only in the characters of its crest; in tube color, sepal color, length of stamens, and in the nature of the appendages of the style it was distinctly intermediate; in sepal length it was not so short as typical *I. fulva* but almost two centimeters shorter than the minimum limit of *giganticaerulea*; when the shape of the petals was examined, this character was found to be typically like that of *I. fulva*. Plant 105 had the same index value as 101 and was identical with it in tube color, sepal color and sepal length and in the nature of its style appendages; in petal shape, however, it was intermediate and not *fulva*-like, and in stamen exertion it was like *giganticaerulea* and not

multiple factors, so that this plant has just a few genes of var. *giganticaerulea*—just enough so that it cannot be classified as a pure *I. fulva*. This plant showed about 76 per cent pollen fertility. Since it is so closely related to *I. fulva* in general aspect, it probably arose as a back-cross of a hybrid (*I. hexagona* var. *giganticaerulea* \times *I. fulva*) to *I. fulva* or perhaps even by a cross of the hybrid from the above cross to *I. fulva*. Table 3 lists the characters of the plants of Colony H1 while, the frequency distribution is expressed in figure 3.

Colony H2.—This colony was growing about fifteen feet farther from the alluvial ridge and therefore from the territory of *I. fulva*. Under these conditions, one might suppose that fewer hybrids and more plants of typical var. *giganticaerulea* would be found in Colony H2. A few clones at the farther end of the

TABLE 3. Analysis of the characters of each plant of Colony H1 with their index values and pollen fertility.

Plant number	Tube color	Color of sepal blade	Sepal length	Petal shape	Stamens	Style appendages	Crest	Index value	Pollen fertility
101	i	RV2	7.0	f	i	i	g	8	76.2%
102	g	VB5	9.6	g	g	g	g	17	93.6
103	i	R3	5.5	f	i	f	i	3	71.9
104	g	BV5	10.2	g	g	g	g	17	95.3
105	i	RV4	7.0	i	g	i	f	8	51.8
106	i	V1	8.1	i	i	g	g	12	93.4
107	g	VB5	9.5	g	g	g	g	17	94.2
108	i	V5	8.6	g	g	i	g	14	84.7
109	i	RV2	8.1	i	i	g	i	10	69.0
110	i	RV2	7.9	g	g	g	i	12	70.3
111	g	VB3	7.6	g	g	g	g	16	92.7
112	g	VB3	7.0	g	g	g	g	15	95.7
113	i	RV2	7.9	g	g	g	i	12	49.2
114	g	VB4	8.7	g	g	g	g	17	89.3
115	i	BV3	8.0	g	g	g	g	15	46.1
116	g	VB4	8.1	g	g	g	g	16	91.8
117	g	VB3	7.6	g	g	g	g	16	95.3
118	i	V2	7.2	g	g	g	g	13	97.8
119	g	VB4	9.3	g	g	g	g	17	94.2
120	i	BV4	7.8	i	g	i	g	13	88.9
121	i	VB4	7.8	g	g	g	g	15	96.7
122	g	V5	9.0	g	g	g	g	16	88.0
123	g	VB3	8.7	g	g	g	g	17	96.4

intermediate; in the nature of its crest it was typically *I. fulva*, whereas plant 101 was identical in this character with the other species. These two plants are very different; yet, when compared with the two species, each considered as a whole deviates quantitatively to the same degree.

Plant 103 remains to be discussed. This plant had no characters of var. *giganticaerulea*. On the other hand, it was not a pure *fulva* as it was intermediate in tube color, stamen length, and form of crest. It was apparently a plant nearer to *I. fulva* but contaminated in three characters by traits of var. *giganticaerulea*. These characters are probably due to

pasture were ignored, so that only twenty-three were examined. Twenty-one of these twenty-three clones had an index value of 16 or 17, so that the expectation of finding a larger proportion of var. *giganticaerulea* than in Colony H1 was realized. The pollen fertility of these plants was 90 per cent or better in all cases except 207 and 209, which had fertilities of only 84 and 52 per cent, respectively. Plant 209 was exceptionally low, as it showed 833 fertile pollen grains to 779 sterile ones. Plants 220 and 223 were undoubtedly of hybrid origin. The former was like *giganticaerulea* in stamen exertion and style appendages and was intermediate in all other characters;

the total index value of this plant was 10, and about 20 per cent of its pollen was bad. Plant 223 had a value of 9 and therefore, as a whole, was half-way between the two species. It was typical of var. *giganticaerulea* in petal shape, of *I. fulva* in the form of the appendages of the style and was of intermediate grade in other respects. The pollen fertility of this plant was only 62 per cent. The individual characters of the plants of Colony H2 are listed in table 4 and the distribution of types in figure 3.

DISCUSSION AND CONCLUSIONS.—The interpretations of Small and Alexander (1931) on the one hand and of Viosca (1935) and Foster (1937) on the other are fundamentally different. There is no dispute as to the existence of many types of *Iris* in Louisiana, but it is difficult to decide which of these types merit specific rank. More than eighty of them are regarded

In a previous section of this paper, the plants of the four colonies of this study were analyzed morphologically. From that discussion and from figure 3, it is readily seen that colonies G and F exhibit only a very small amount of variation within themselves. The plants of Colony G were remarkably alike and all were pure specimens of *I. hexagona* var. *giganticaerulea*. Colony F was similarly uniform in morphological appearance and consisted exclusively of plants of *I. fulva*. However, Colony H1 is strikingly different from either of these two, as the plants that form this colony are not all alike, nor are the differences small ones. Only ten of the twenty-three plants were typical of *I. var. giganticaerulea*, and since no plants scored 0 or 1, the other thirteen were intermediate in some degree in their total index values. The three plants that scored 15 deviated only slightly

TABLE 4. Analysis of the characters of each plant of Colony H2 with their index values and pollen fertility.

Plant number	Tube color	Color of sepal blade	Sepal length	Petal shape	Stamens	Style appendages	Crest	Index value	Pollen fertility
201	g	VB4	9.6	g	g	g	g	17	98.6%
202	g	BV5	10.9	g	g	g	g	17	93.0
203	g	BV4	10.0	g	g	g	g	17	99.2
204	g	VB5	9.4	g	g	g	g	17	92.7
205	g	VB4	10.2	g	g	g	g	17	97.3
206	g	BV5	9.0	g	g	g	g	17	89.9
207	g	BV5	7.5	g	g	g	g	16	83.7
208	g	BV4	9.5	g	g	g	g	17	90.2
209	g	VB5	9.7	g	g	g	g	17	51.7
210	g	BV5	9.0	g	g	g	g	17	96.7
211	g	VB4	9.0	g	g	g	g	17	95.3
212	g	BV4	9.0	g	g	g	g	17	89.6
213	g	VB4	9.1	g	g	g	g	17	95.3
214	g	VB4	9.1	g	g	g	g	17	91.9
215	g	VB5	9.5	g	g	g	g	17	95.1
216	g	BV4	9.8	g	g	g	g	17	93.4
217	g	VB3	9.8	g	g	g	g	17	98.3
218	g	VB5	9.2	g	g	g	g	17	97.8
219	g	VB3	8.9	g	g	g	g	17	91.6
220	i	RV2	7.3	i	g	g	i	10	79.5
221	g	BV3	9.2	i	g	g	g	16	95.9
222	g	VB5	9.6	g	g	g	g	17	94.1
223	i	V2	7.1	g	i	f	i	9	62.2

as species by Small and Alexander, but Viosca considers that only four types are valid species, that some of the others are merely geographic races or varieties of these four species and that most of the types are hybrids between *I. hexagona* var. *giganticaerulea* and *I. fulva*. Foster's treatment is basically the same as that of Viosca. The present paper does not deal directly with any of Small and Alexander's "species." It does, however, study the problem of hybridization in general in these irises to learn whether hybrids are abundant, whether they are found in the regions of most of Small's "species," and whether there would be good reason to suppose that these "species" could be regarded as hybrids.

from *giganticaerulea*, but not even these were all alike. These plants might have been of the nature of (*giganticaerulea* × *fulva*) × *giganticaerulea* or even a further cross of this hybrid to the blue-flowered parent. The six plants that scored 12, 13, or 14 had obviously more *fulva* "blood," and only two of these six were alike. Such plants surely suggest hybridization and appear to be segregates, possibly from a backcross of the F_1 to *giganticaerulea*. Plant 109 scored 10 and was closer to *I. fulva*, while 101 and 105 were intermediate in total score; these could not have been F_1 plants, as they were very different from one another and were probably segregates of the F_2 or later generations or arose as a

back-cross of an F_1 or F_2 plant to one of the parents. Plant 103 was close to *I. fulva*, although it was intermediate in three characters. These thirteen plants might be considered additional species, but their morphological analysis would equally well indicate that they were hybrids. The character analysis shows that they possess no new characters which are absent in both *giganticaerulea* and *fulva*; all the characters of these plants resemble those of either of the two species or are intermediate between them; various plants show different recombinations of the characters of *fulva* and *giganticaerulea*. These facts indicate that the hybrid origin of these plants is more plausible than the assumption that each type is a different species. Colony H2 also contains two plants which may be regarded as being hybrids for the same reasons as the thirteen variant plants of Colony H1. On the basis of morphological evidence alone, these plants which are neither *fulva* nor *giganticaerulea* may well be regarded as hybrids. It might be well, at this point, to examine the problem from the point of view of ecology.

Although these two species may often be found within a few feet of one another, each is restricted to a definite habitat. This is not appreciated from Small and Alexander (1931) who state that *giganticaerulea* is found in "marshes, meadows, ditches and pools," while *fulva* is present in "swamps, marshes, bayou-banks and ditches." Actually, *I. fulva* is definitely restricted to land of deltaic origin which has been formed by alluvial deposits from present or former distributaries of the Mississippi River. Such alluvial land must also be sufficiently far above sea level so that it is not covered nor even sprayed with salt water during severe storms, as this species will not tolerate much salinity. For that reason, *I. fulva* is never found near the Gulf. This species also prefers water with a pH about neutrality and is almost always found in a shady situation. In the alluvial land, *I. fulva* may be found chiefly in three regions: (1) it is found in abundance on the banks of present or abandoned deltaic streams in the clayey soil, on the edge of the alluvial ridge adjoining the stream; (2) if the deltaic stream itself was cut off some time ago and filled up, it may contain plants of *I. fulva* if it is sufficiently shallow; (3) as the ridge proper slopes away from the bank, it will not contain *I. fulva* until its far edge is reached; if this edge borders on a cypress swamp, this species will be present in the shallowest part of the swamp, at the edge. This habitat is definitely a restricted one. *I. hexagona* var. *giganticaerulea* may or may not be found in land of deltaic origin. It is a marsh-loving species and is found in fresh-water marshes near the level of the Gulf of Mexico, no matter what was the origin of the marsh. It is tolerant of a fair degree of salinity and is found in sunny areas chiefly. It grows in black, mucky soil, and prefers a region where the water level is continually fluctuating due to "wind tides." This species does not thrive in well wooded places, while *fulva* prefers the woody

banks of deltaic streams or the deep shade of the border of a cypress swamp. These ecological relationships have been discussed by Viosca (1935) and corroborated by the present author on numerous occasions.

This restriction of species to habitat is a rigid one, and the two species are very effectively separated by this ecological barrier; but under certain conditions apparently the barriers may be broken down and the two species may come sufficiently close together to produce hybrids. It is at this point that the hand of man is seen to have an influence, for spheres of contact intimate enough for hybridization are not common under undisturbed natural conditions, although they have been found in a few instances. In general, the role which man plays in establishing regions where two species can produce hybrids is two-fold. The first effect of man is to lower the ecological or geographic barrier which effectively keeps the two species apart. Once this separation in space is eliminated, the two species may come into contact, and if no genetic barrier is present, they may hybridize. The second part of man's role is to create new niches where the hybrid seeds can germinate and develop into fine, healthy plants which later flower and produce seeds. Unless the soil is prepared so that it will house these intermediates, the hybrids would never become established, and the break-down of the barrier would have little effect. Viosca (1935) has described hybridization of *Iris* in such a region. On the Lafitte ridge south of New Orleans, *fulva* is found in the woods, while *giganticaerulea* is restricted to mucky depressions adjoining the woods and never penetrates them. Normally the two species are never sufficiently near one another to hybridize. However, at certain points the effect of human agencies is seen. Where man has cleared the woods at the edge, the barrier is removed and the blue-flowered species can penetrate into the *fulva* territory which is no longer too shady to sustain it. When the contact has thus been intimately established, the two species can intercross and produce hybrid seeds, since there is no chromosomal or genetic barrier to hybridization between these species, as the production of artificial hybrids demonstrates. Man has also allowed cattle to graze on this cut-over woodland, and cattle have the effect of fertilizing the land, cultivating the soil, thinning out the competitors of *Iris* and generally making the soil well suited for the growth of hybrid *Iris* plants. In this region near Lafitte, a number of forms have been found which have been considered hybrids by Viosca. If the two species were to differ one from the other by a large number of genes, an almost limitless number of different kinds of hybrids might be produced at such zones of contact. When all the possible F_2 and F_3 segregates are considered as well as back-crosses of the F_1 and later generations to either parent, a very large number of possible types is revealed. At such zones of contact, many small, rhizome-propagated clones would be expected, and almost all these clones

would differ at least slightly from the rest, since the effects of some of the gene-differences would be slight.

It has been shown that some of the plants of Colonies H1 and H2 can be regarded as hybrids on the basis of their morphological characters and total index values, and it is readily seen from figure 1 that these two colonies are situated geographically between the habitats of the two species. The ecological relationships of these colonies may now be considered. A large stand of *I. hexagona* var. *giganticaerulea* (Colony G) was located in the mucky soil of a marsh at the side of Bayou Boeuf. This bayou cuts across what was once a deltaic stream, the outlines of which can be traced, as the level of this former distributary of the Mississippi is still from several inches to a foot or two below the level of the deltaic ridges which outline its course on either side. This abandoned deltaic stream had built up typical alluvial ridges on each side of it which were highest near the stream and then tapered off a considerable distance into cypress swamps. When the stream was cut off as a distributary, it filled up gradually but never to the height of the ridges. These ridges tended to isolate bodies of water without an outlet, and apparently this water finally broke through and cut across the ridge almost at right angles, forming the present swamp drainage bayou, Bayou Boeuf, which is not full of silt and does not lay down alluvial ridges along its bank, but forms marshes at certain points. Bayou Boeuf connects two lakes, and the direction of its current may be reversed by "wind tides," so that its height may change considerably from time to time, and thus the water level of the adjoining marshes may fluctuate greatly. The mucky soil of a fresh water marsh with a fluctuating water level and situated in the open in a sunny location forms an ideal habitat for *giganticaerulea*. Such is the location of Colony G in a marsh beside Bayou Boeuf. This colony is really in the bed of the former deltaic stream at the point where the abandoned stream is intersected by the swamp drainage bayou. Apparently, when Bayou Boeuf cut through the alluvial ridges and connected the two lakes, *giganticaerulea* in the form of rhizomes or seeds floated down it or was washed along it by the wind tides, and some plants became established in the marsh at the intersection. As conditions were ideal, a large colony finally became established there.

Along the alluvial ridge at one side of the abandoned stream runs a road. The ridge on the other side is generally wooded but cleared at the edge of the former stream to make room for a group of houses and yards. On the bank of the ridge and extending back into the woods for a distance were some plants of *I. fulva*. At this edge of the bed of the former stream they were not very numerous because this region was used as a yard, but some were present coming right down to the edge of the former stream. The plants which were found near the bed of the old stream and those observed in the woods were typical *I. fulva*, but for this character analysis,

plants of another part of the ridge several miles away were selected and described as Colony F. Both groups of plants were pure *I. fulva* from the same habitat in the same territory, and the plants used for measuring were more accessible. The situation then is that of a former deltaic stream with *I. fulva* growing on the banks of the alluvial ridge intersected by a swamp drainage bayou which has carried *giganticaerulea* to the place of intersection.

Beginning a couple of hundred feet from the intersection on the same side of the road as the deltaic stream is found, is a row of small houses and yards located at the highest point of the alluvial ridge. The wooded part of the ridge around the houses is generally cleared, and the land between many of the houses and the road is also mostly cleared with the exception of a few cypress trees. This land is generally used to pasture cattle. Before one of these houses about 500 feet from the intersection of the former deltaic stream and Bayou Boeuf and, therefore, about that distance from Colony G were located Colonies H1 and H2. The latter colony was exactly in the center of the bed of the abandoned stream, while Colony H1 was nearer to the bank of the ridge and therefore nearer to *I. fulva*. It must be remembered that Colony H2 was almost pure *giganticaerulea* with two important exceptions, and that Colony H1 contained thirteen plants which could be interpreted morphologically as hybrids. If the morphological interpretation is correct, it is not difficult to see how hybridization could have arisen. The *fulva* plants on the bank of the old deltaic stream are in typical country and have probably been there a considerable time. When Bayou Boeuf cut its channel, *giganticaerulea* plants became established in the marshes on its edges, including the marsh at the intersection. These plants may not be so long-established as those of *I. fulva*, but are probably not very recent. No plants of *I. fulva* grew in the channel of the former stream because it was not sufficiently shallow, and no plants of *giganticaerulea* became established there until relatively recently, because until about ten years before this study, the land had not been cleared and used for pasturing. About 1926, human agencies came into play and changed the ecological situation. People who are familiar with the country maintain that none of the unusual types were found before that time, and it was only then that the land was cleared and turned over to cattle, and drainage canals were cut, which caused the formerly steady water level to fluctuate. These factors made the land suitable for the growth of var. *giganticaerulea* and thus effectively broke down the barrier which had previously kept these two species from coming sufficiently close together to intercross. High wind tides shortly thereafter washed a few rhizomes of *giganticaerulea* down the bed of the former deltaic stream, and they remained there, becoming established in the center of the stream in land now for the first time suitable for their growth. That they should occupy the center of the stream bed first is natural. From

that area some seeds probably fell towards the side and established colonies of *giganticaerulea* nearer the edge of the alluvial ridge. With *giganticaerulea* established in the bed of the stream and within a few feet of *I. fulva*, crossing was easily accomplished. Not only had man broken down the barrier, but by clearing the land and allowing cattle to graze in it, he had also made it suitable for sustaining the hybrids. Therefore, Colony H2, since it was at the center of the former stream, contained mostly *giganticaerulea* but also two hybrids, and Colony H1, which was nearer to *I. fulva*, contained a higher proportion of hybrids. The ecological and geographical considerations thus bear out the morphological evidence that the exceptional plants of Colonies H1 and H2 are of hybrid origin.

The only alternative to the hypotheses of origin by hybridization is that the plants which differ from both *fulva* and *giganticaerulea* are distinct species. If they are different species, they must have evolved in that particular area very recently or have immigrated into the region within the past twelve years. All local information, much of which is reliable, is that these new types were definitely not there twelve years ago. The cutting of drainage channels and the opening up of the land are sufficient to break down a barrier to crossing, and in view of all the evidence, it is more plausible to assume that hybridization occurred suddenly once the only barrier to it was removed than to believe that an unusually large number of types suddenly was transported there from an undetermined source or that a wild burst of mutations suddenly occurred. The best way to regard the areas of Colonies H1 and H2 is that they consist of some clones of *giganticaerulea* washed in from the marsh, other clones of this variety which developed from self seed of the plants washed in, and a number of hybrid clones which today must be twelve years or less in age. Just at the edge of the colony are some clones of *I. fulva* on the slope of the alluvial ridge.

This case resembles in many respects the conclusions which Wiegand (1935) has reached in regard to hybridization: a series of observations upon the genus *Amelanchier* has led him to assume that natural hybrids would "not ordinarily present any new characters" but would show recombinations of parental characters and that some characters might be intermediate. He believes that the range of the hybrids would be infinitely smaller than the ranges of true species and that the hybrids would not be found outside the range of the two supposed parents. The hybrid types of Colonies H1 and H2 agree with all these requirements. Wiegand further concludes that a great burst of crossing may occur locally all at once due to disturbances of the environment. Apparently no hybrid types existed in the area of Colonies H1 and H2 until the change in the water level of the region and the opening of the pasture to cattle permitted crossing in that particular local area. While this crossing was probably sudden, it

was not extensive; on the other hand, more varieties of types can be found in this one small area than will be observed in many square miles of typical *fulva* or *giganticaerulea* territory.

If these plants are to be considered hybrids, what light do they throw upon the hybrid origin of Small's numerous "species"? No attempt was made to identify these plants with any of Small's "species," chiefly for two reasons. In the first place, as Viosca (1935) has pointed out, Small's keys are hardly usable because they are very vague on many points. Secondly, if hybridization occurs, as this study shows, a very large number of types would be produced; if the two species differed by as few as ten unlinked genes, over a thousand possible combinations of F_2 plants would result, while Small and Alexander (1931) list only about 80. The chance of finding any one of the 80 out of all the possible types is so small as to make a comparison of hybrid plants with Small's types a burden which is not justified by the possibilities of success. Many of Small's "species," however, bear certain striking resemblances to the hybrids of Colony H1 and H2. In the first place, morphologically they can be interpreted as intermediates between *I. fulva* and *I. hexagona* var. *giganticaerulea*, for, like the hybrids of this study, their characters appear to be recombinations of those of these two species or are intermediate between the two species, and these plants never show any new traits. Secondly, they have all been found in regions which are situated geographically between the two species; none, for example, has been found in a region where one of the two main species is absent. Thirdly, they have always been found in an ecologically intermediate habitat; they have been discovered in regions ecologically like the abandoned deltaic stream of this study—areas which have been disturbed by man and in which the natural ecological barrier has been eliminated. Another factor which points to the hybrid origin of many of the types which Small and Alexander list as species is their resemblance to artificially produced hybrids. Hybridization work has not been extensive between these two species. Viosca (1935) reports instances of artificial cross-pollination by Martin Burkenrood and by himself and cites some hybrids that appeared spontaneously in the garden of Miss Caroline Dornon. Reed (1931) reports an interesting cross between *I. fulva* and *I. brevicaulis* Raf. which he has carried into the F_2 generation. The F_2 plants form an almost endless variety of types, showing the same kinds of recombinations of characters and of intermediate characters that the present study shows exist in nature when two species of *Iris* are crossed and which correspond in a striking manner to the recombinations which form many of Small's "species."

SUMMARY

Four colonies of *Iris* in southeastern Louisiana were examined for evidences of hybridization between *I. fulva* Ker-Gawler and *I. hexagona* var. *giganticae-*

rufes (Small) R. C. Foster. Twenty-three plants were studied in each colony.

Seven characters were examined on each plant; they were tube color, color of the blade of the sepal, sepal length, petal shape, length of stamens, shape of style appendages, and form of the crest.

Arbitrary values were assigned to each character; if like *I. fulva*, the value was 0; if like *giganticaerulea*, it was 2, 3, or 4, depending upon the character, and if intermediate, it was between 0 and the value assigned to *giganticaerulea* for that character. The total of the values of all the characters of a plant represents the index value of that plant. The total index values of all the plants of each population are plotted in a histogram in order to compare the four colonies.

Colony G was pure *giganticaerulea*, and Colony F was pure *fulva*. In Colony H2, two plants were undoubtedly hybrids between these two species, while the remaining twenty-one plants were pure *giganticaerulea*. In Colony H1, ten plants were of this species, while thirteen were hybrids of varying degrees of hybridity.

Colonies H1 and H2 were situated geographically between the two pure species. Further, they were in an area which had been disturbed by man. This had broken down the ecological barrier which normally separates the two species and allowed them to mix. It further provided land which was suitable for sustaining hybrids, so that once formed, they became well established.

I. fulva is restricted to deltaic land, usually on the sides of deltaic ridges, while *I. hexagona* var. *giganticaerulea* is found in fresh water marshes. They normally do not come close enough together to cross,

but this may occur when human agencies disturb natural conditions. In such a region the hybrids of this study were found and in such regions many of Small's "species" have been found.

Many of the types considered species by Small show the same relation to the two species morphologically, geographically, and ecologically that the hybrids of this study exhibit and are probably themselves hybrids.

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NUCLEAR SIZE IN PLUMULAR MERISTEMS OF INBRED AND HYBRID MAIZE¹

Elizabeth A. Bindloss

ALTHOUGH VARIOUS theories have been suggested in explanation of hybrid vigor or heterosis, the problem is by no means adequately solved. Jones' (1917) genetical interpretation of heterosis as due to linked dominant genes has been generally accepted. However, East (1936) and others have called attention to weaknesses in it, notably its assumption that factors for large size are always dominant over those for small size.

Ashby (1930, 1932) has studied heterosis in maize from a physiological viewpoint. He found no differences between parents and hybrids in cell size, photosynthetic efficiency of leaves, or time of flattening of the sigmoid growth curve. The true growth rate of the hybrid, though greater than that of one parent, was the same as that of the other and appeared to

be an inherited trait. The only fundamental difference, other than a higher percentage of germination in the hybrids, was that the hybrid embryo was larger and heavier than that of either of its parents and so germinated with an initial advantage which was maintained throughout the life-cycle. Ashby explains heterosis on the basis of greater meristematic mass in the dormant embryo of the hybrid.

A histological study of the plumular meristems in such embryos should show whether differences in initial "capital" of the embryo as a whole are due primarily to differences in size relationships at the growing point itself. The purpose of the present paper is to compare the plumular meristems in the embryos of inbred lines of maize with those of their hybrids, especially as to the size of their nuclei, and to note any differences in the meristematic region within these lines and hybrids which might bear on the problem of heterosis.

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In a comparison of meristematic mass in the hybrids with that of their parents only estimates are possible, since irregularity of shape makes an accurate quantitative measure of size impossible. Similarly, cell size determination cannot well be made because of the variability in cell shape at the meristem. Nuclear size, however, is readily measured and has the advantage of presumably being related to the rate of metabolism within the cell. A study of nuclear size at the growing point might thus be expected to throw some light on the size and rate of growth of the plant developing from it.

MATERIALS AND METHODS.—The parents and hybrids designated as Pedigree 1 were obtained through the courtesy of Dr. J. H. Kempton of the U. S. Department of Agriculture. They consisted of the inbred parents "Pawnee" and "Pipe" together with their reciprocal crosses, and came from the same stocks as those investigated by Ashby (1930) in his first study of heterosis in maize. Ashby's notation for the parents has been retained in discussions of this material. Thus Pawnee, referred to as P_w , is a blue-kerneled strain with starchy endosperm which has been inbred for 18 generations. Pipe, referred to as P_p , has white kernels with starchy endosperm and has been inbred 13 generations. The two parents are distantly related varieties. The degree of relationship of the parents in the other pedigrees is unknown.

Pedigree 2, consisting of two inbred lines of Leaning corn and their reciprocal crosses which are heterotic, was obtained through the courtesy of Dr. D. F. Jones of the Connecticut Agricultural Experiment Station. These lines, designated as Leaning 236 and Leaning 243, are referred to here as P_1 and P_2 ,

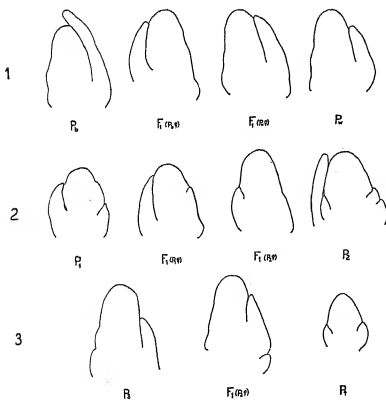


Fig. 1. Median longitudinal sections through the plumular meristems of typical dormant maize embryos. 1, Pedigree 1; 2, Pedigree 2; 3, Pedigree 3. $\times 86$.

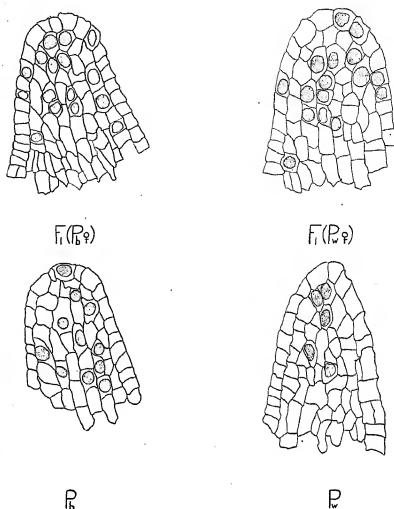


Fig. 2. Median longitudinal sections of the plumular meristems of a typical dormant maize embryo from each inbred line and reciprocal cross of Pedigree 1. Drawn with the aid of a camera lucida. $\times 300$.

respectively. They resemble each other in having yellow kernel color and starchy endosperm.

Pedigree 3 consisted of two inbred lines, 228-6-5 and 228-4-8, and their hybrid (228-6-5) \times (228-4-8), supplied by Dr. G. F. Sprague to whom the writer is indebted. These lines were later inbreds of those used by Ashby (1932) and by Sprague (1936). Both parents had yellow kernels and starchy endosperm. Line 228-6-5 is referred to as P_3 and line 228-4-8 as P_4 .

The meristem was selected as the most favorable region for making a comparison of nuclear sizes, since it provides strictly comparable data for individuals within lines and between lines, as there is little differentiation of tissue at this point and a minimum of vacuolation in cell and nucleus.

In order to measure nuclear size in the plumular meristem, the embryo was dissected from the endosperm after soaking the kernels in water for 12 hours. At the end of that time the endosperm was soft enough to be removed, leaving the scutellum still attached to the embryo. The tip of the coleoptile and the enclosed leaves were cut off so that the presence of air between the primary leaves would not prevent rapid killing of the material and infiltration with paraffin. The material was fixed in "Craf" (Randolph, 1935) and run into paraffin by the butyl-

TABLE 1. Mean nuclear volumes in plumular meristems of maize embryos.

	Total number of nuclei	Mean volume in cubic micra	Size relative to smaller parent
Pedigree 1			
P _b selfed	552	372 ± 5.1 ^a	100
P _w selfed	271	518 ± 11.2	139
P _b (♀) x P _w	190	523 ± 11.2	140
P _w (♀) x P _b	592	596 ± 8.5	160
Pedigree 2			
P ₁ selfed	174	334 ± 7.3	100
P ₂ selfed	447	506 ± 7.6	151
P ₁ (♀) x P ₂	457	454 ± 6.1	136
P ₂ (♀) x P ₁	329	425 ± 7.2	127
Pedigree 3			
P ₁ selfed	362	441 ± 7.5	100
P ₂ selfed	312	510 ± 8.0	116
P ₂ (♀) x P ₁	376	517 ± 8.3	117

^a Standard error.

TABLE 2. Test for significance of difference in mean nuclear volumes.

Compared strains of maize	Difference of mean volumes $M_1 - M_2$ D	Standard deviation of difference ^a σ_D	D/ σ_D	
Pedigree 1				
P _w - P _b	146	12	12	significant
F ₁ (P _b ♀) - P _w	5	16	.3	not significant
F ₁ (P _w ♀) - P _w	78	14	5	significant
F ₁ (P _b ♀) - P _b	151	12	13	significant
F ₁ (P _w ♀) - P _b	224	10	22	significant
F ₁ (P _w ♀) - F ₁ (P _b ♀)	73	13	5	significant
Pedigree 2				
P ₂ - P ₁	172	10	17	significant
P ₂ - F ₁ (P ₂ ♀)	81	10	8	significant
P ₂ - F ₁ (P ₁ ♀)	52	10	5	significant
P ₁ - F ₁ (P ₂ ♀)	91	10	9	significant
F ₁ (P ₁ ♀) - F ₁ (P ₂ ♀)	29	10	2.9	significant (?)
Pedigree 3				
P ₂ - P ₁	69	11	6	significant
F ₁ (P ₂ ♀) - P ₁	76	11	7	significant
F ₁ (P ₂ ♀) - P ₂	7	11	.6	not significant

$$^a \sigma_D = \sqrt{(\sigma M_1)^2 + (\sigma M_2)^2}$$

alcohol method. Longitudinal sections of the embryos were cut at 10 μ and stained in haematoxylin.

Cumera lucida drawings were made of the nuclei in the plumular meristem of median sections under oil at a projected magnification of 1100 \times . Selection of spherical nuclei reduced the degree of error incurred in determining the mean diameters, which were measured to the nearest quarter of a millimeter from the drawings. To insure comparable data on nuclear size, only nuclei within the meristem were selected within the region from the apex to the first leaf pri-

mordium. Nuclear volumes were determined on the basis of a sphere. Approximately 10 to 14 embryos were studied from each line and each cross, the number of nuclei measured within a particular type varying from 200 to 600. Larger numbers were not obtained, owing to the fact that relatively few spherical nuclei were available in each median section of a meristem.

The standard error of the difference between grand means was used as the basis for determining the significance of the differences between populations

together with an analysis of the variance (Snedecor, 1934) which makes allowance for the variability within, as well as between, these populations.

RESULTS.—Meristem and cell size.—The plumular meristems of the embryos were briefly observed. Camera lucida drawings of the plumular meristems in the embryos showed that there were no striking differences between the size and shape of the meristems in median sections in the parents and hybrids of Pedigrees 1 and 2. This is best seen in figure 1 which shows typical individuals from each line and cross of all three pedigrees. On the other hand Pedigree 3 was unique in having the meristem of one parent (P_1) much smaller than that of the other parent and its hybrid.

Difficulties in measuring cell size make definite conclusions regarding such size relationships of the pedigrees impossible. However, observations of drawings (fig. 2, 3) of the cells in Pedigrees 1 and 3 indicate that cell size is essentially the same for both inbreds and hybrids.

Nuclear size.—Table 2 shows that in Pedigree 1 a comparison of mean nuclear volumes by means of the standard error of the difference of the means indicates that the two parents differ significantly from each other. One hybrid has nuclei significantly larger than either parent and the other hybrid. The other hybrid has nuclei significantly larger than one parent, but not the other. Table 1 shows that when the mean

size of the nuclei in P_1 is taken as the base, parent P_2 is greater by 39 per cent; the hybrid with P_1 as its female parent is greater by 40 per cent; the other hybrid, with P_2 as the female parent is greater by 60 per cent. Maternal influence on nuclear size is suggested by this difference in the sizes of the nuclei in reciprocal crosses. In both hybrids the nuclear volume was as great as or greater than the parent (P_2) which had the larger nuclei. Analysis of the variance substantiates the reliability of these data based on the grand means of the pedigree. This pedigree suggests that there are larger nuclei in the plumular meristems of hybrid embryos than in their inbred parents.

In Pedigree 2, however, hybrid nuclei are no greater in volume than those of the parent with larger nuclei. The nuclear volume of parent P_2 is approximately 51 per cent greater than P_1 , and the hybrids occupy an intermediate position between these two parental extremes. Calculation of the standard error of the difference of the means (table 2) shows that the parents are significantly different in nuclear size, that the hybrids differ significantly from both parents, but not from each other. This conclusion is substantiated by an analysis of variance. Since the difference in the volumes of the reciprocals was not significant, the female parent does not seem to be important here in affecting nuclear size. Heterosis is clearly not related to greater nuclear size in this pedigree.

Although nuclear volumes of parents P_3 and P_4 of Pedigree 3 and those of parent P_3 and the hybrid differ significantly, nevertheless tests for variance indicated that the amount of variation within each line and hybrid was so great that the grand averages based on the measurements were not reliable.

The grand mean nuclear volumes for each inbred and hybrid of the three pedigrees are shown graphically in figure 4. The constancy of nuclear sizes for each inbred line and hybrid suggest that nuclear size is an inherited trait.

DISCUSSION.—The presence of relatively large plumular meristems in embryos of maize plants which show hybrid vigor might be expected from Ashby's theory that heterosis is due to more capital in the hybrid embryo rather than to physiological differences. Such a correlation of large meristem size and organ development has been found in *Sequoia gigantea* by Buchholz (1938) where there is a relationship between the size of the promeristem and the size of the twig which it initiates. However, in the three pedigrees of maize here studied, size and vigor of the plants known to be heterotic seemed to be independent of plumular meristem size. The small degree of variability in meristematic shape and size for each inbred and hybrid suggested that these characters were inherited.

Although the total meristematic mass at the growing point as observed in these pedigrees is thus not related to the degree of vigor shown by a plant, nevertheless the plant's future development may be indicated by nuclear size in the meristematic cells.

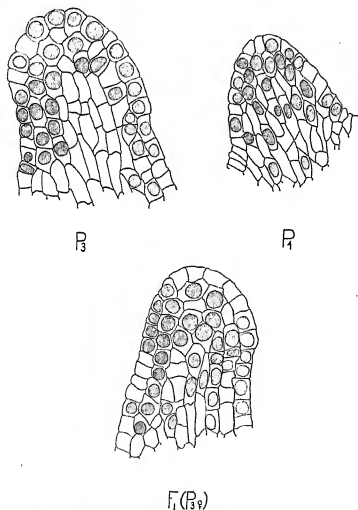


Fig. 3. Median longitudinal sections of plumular meristems of a typical dormant maize embryo from each inbred line and the hybrid of Pedigree 3. Drawn with the aid of a camera lucida. $\times 300$.

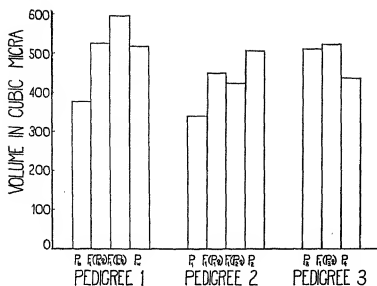


Fig. 4. Nuclear size relationships in the plumular meristems of dormant maize embryos from three pedigrees consisting of two inbred lines and their hybrids.

Various workers have shown a relationship between nuclear size and cell metabolism. This greater nuclear size may be observed in such active cells as the glandular cells in animal tissue and secretory cells or hairs in plant tissues. Thus Yampolsky (1937) in a developmental study of the ovarial trichomes of *Mercurialis annua* found that in these hairs the nucleus became many times the size of those in the epidermal cells from which the trichomes developed. Fischer (1934) observed that near wounds in leaves of *Peperomia blanda* the nuclei were much larger and that they frequently began to divide very rapidly. There was an increase in chromatin associated with nuclear swelling. These facts indicate a correlation of greater nuclear size with increased metabolic activity in tissues adjacent to regions of wounding. Although there is little direct evidence, the nucleus is generally regarded as the center at which the enzymes coordinating and regulating cellular metabolism are produced.

Evidence that larger plants have larger nuclei and are more vigorous is also furnished by studies in polyploidy. Müntzing (1936), in a review of the work on autopolyploidy and its evolutionary significance, observed that the majority of autotetraploids were *gigas* forms and that this character ultimately depended upon increased cell size which is in turn related to increased nuclear size. Gates (1909) found in *Oenothera gigas*, a tetraploid mutant from *O. lamarckiana*, that the increased size of the *gigas* plant could be traced to increased nuclear and cell size in the various organs. Similar observations have been made by other investigators.

The presence of greater nuclear volumes in bivalent and trivalent forms of a polyploid series of *Dianthus* has been shown by Rohweder (1934). He noted also that these forms with greater nuclear volumes were more vigorous than the univalent plants. Studies of the geographic distributions of plants have shown

that the part of the flora thriving under the most difficult conditions of nutrition and climate possesses a higher percentage of polyploids (Rohweder, 1936, 1937), suggesting again the greater physiological vigor and adaptiveness of forms with increased bulk of chromatin and thus larger nuclei.

In the maize material here studied, however, no consistent correlation can be observed between greater nuclear size and heterosis. Such a correlation seems to be indicated by the large nuclei of the hybrids in Pedigree 1. However, Ashby (1930) showed for this material that parent P₁, which was found here to have smaller nuclei, had the greater growth rate. Thus vigor in the parent is apparently associated with small rather than large nuclear volumes at the growing point. Pedigrees 2 and 3 show no relationship between heterosis and nuclear size, since in the former the hybrids were intermediate in size between the parents, and in the latter the hybrid was the same size as the larger parent.

SUMMARY

A study was made of the plumular meristems and their nuclear volumes in the embryos of three maize pedigrees, each consisting of two inbred lines and their hybrids. In each case the hybrids had been found to display heterosis.

There was no correlation between heavier hybrid embryos, as reported by Ashby, and larger plumular meristems in these embryos. The size of the meristem in the hybrids appeared no greater than that in the larger parent. In one pedigree, the two parents differed greatly in the size of their meristems, but the hybrid meristem was no larger than that of the larger parent. There was no apparent correlation of greater meristematic mass with increased vigor in the hybrids.

In general, nuclear size in the plumular meristem was relatively constant for each inbred line and each hybrid, suggesting that it is an inherited trait.

In all three pedigrees the nuclear volumes of the parents differed significantly, but the reliability of the data in one pedigree is questionable, due to great variability within the lines and hybrids of that pedigree.

In one pedigree the nuclei of the reciprocal hybrids were significantly larger than those of one or of both parents. In a second and genetically distinct pedigree, however, the hybrids had nuclei intermediate in size between those of their two parent lines. Mean nuclear volume in a hybrid was never as small as in its smaller parent.

A positive correlation between nuclear size and heterosis in the hybrids is therefore suggested in one pedigree, but in the others no such correlation could be demonstrated.

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BERBERINE AS A FACTOR IN THE RESISTANCE OF MAHONIA TRIFOLIOLATA AND M. SWASEYI TO PHYMATOTRICHUM ROOT ROT¹

Glenn A. Greathouse and G. M. Watkins

MAHONIA TRIFOLIOLATA Fedde (*Berberis trifoliolata* Moric.) and *M. Swaseyi* Fedde (*B. Swaseyi* Buckl.), which occur in the Edwards Plateau region of central Texas, are often observed in areas known to be infested with the cotton root rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar. These shrubs are not attacked under such conditions and are generally considered resistant (Taubenhaus and Ezekiel, 1936). Both species have been studied from this standpoint by Mr. S. E. Wolff and Dr. C. H. Rogers in the root rot test nursery at Temple, Texas, and found to be highly resistant or immune.

In a recent paper (Greathouse, 1938) attention was directed to the presence of alkaloids in the roots of a number of plants known to be resistant to *Phymatotrichum* root rot with the suggestion that such organic nitrogen bases may be an important part of the mechanism of resistance in those plants. The occurrence of the alkaloid, berberine, in the various natural orders of the plant kingdom has been sum-

marized by Henry (1924, p. 208) and by Allen (1929, p. 267), who state that it is frequently found in association with other alkaloids, such as berbamine, oxyacanthine, hydrastine, canadine, etc. Berberine is the principal alkaloid in the root tissues of *Mahonia trifoliolata*, according to Hart (1916), who found 1.49 per cent of berberine and about 0.1 per cent of associated alkaloids; hydrastine was absent, however. Richter (1914), Cromwell (1933), and others have determined gravimetrically the berberine content of a number of species of the barberry family. Although microchemical studies by a number of workers on *Berberis vulgaris*, as summarized by Goris (1914) and by Cromwell (1933) on *B. Darwinii*, have shown the distribution of berberine in root tissues of these two species, the writers are unaware of any microchemical work on *M. trifoliolata* and *M. Swaseyi*, or of any previous studies of the alkaloid constituents of the latter species. The present investigation was undertaken to determine the quantities of berberine in the various organs of these plants, to study the distribution of the alkaloid in the root tissues, and to determine the effect of berberine on the growth of *P. omnivorum* in pure culture.

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MATERIALS AND METHODS.—Typical specimens of *M. trifoliolata* were excavated in the field in several localities in Travis, Brown, and Bell Counties in central Texas, and *M. trifoliolata* and *M. Swaseyi* were obtained from the root rot nursery at Substation No. 5, Texas Agricultural Experiment Station, Temple, Texas.² The excavated plants were brought directly to the laboratory, cut up into pieces, and separated into lots in such a way that all parts of a given size were in one group. This resulted in several different lots of roots, stems, leaves, and fruits. Very young roots of *M. trifoliolata* were obtained from bushes which had been transplanted and re-excavated after 67 days. All lots of tissue were dried at 80°C. under 3 to 4 cm. pressure, ground to pass a 60-mesh sieve, and again dried to constant weight.

The following method was used for the isolation of berberine from roots of *M. trifoliolata*. Two kilograms of root tissue were thoroughly extracted with warm 95 per cent ethyl alcohol and the solvent largely distilled under reduced pressure. Much hot water was added and the mixture rendered acidic to Congo red with hydrochloric acid. The remainder of the ethanol was removed by means of reduced pressure and the mixture cooled to allow the hydrochlorides of the alkaloids to crystallize. The resulting crystals were removed and purified either by re-dissolving in alcohol and precipitating with ether or by dissolving the crystals in a mixture of 1000 gms. of water and 500 gms. of acetone. The mixture was made alkaline with sodium hydroxide, and the lemon yellow acetone compound which precipitated was further purified by boiling in 50 ml. of dry alcohol containing 5 ml. of chloroform. Following either of these procedures for crystallization and purification of berberine, a small amount of syrup of alkaloidal nature remained, from which no crystalline substances were obtained.

For the determination of the concentration of berberine in the various parts of *M. trifoliolata* and *M. Swaseyi*, 5 to 10 gms. of the powdered tissue were extracted with ethyl alcohol in a Soxhlet apparatus until the extract gave no further test for alkaloids, and the berberine was estimated in the alcoholic extract by the method of Richter (1914), which is based upon the fact that berberine in one of its isomeric forms is soluble in ether. The normal or crystalline form (ammonium form) is soluble in water and insoluble in ether; by the action of alkali this is converted into the presumed "aldehyde form" which is soluble in ether and practically insoluble in water. The method is as follows: The alcoholic extract was evaporated to dryness on a water bath and the residue taken up with hot water, which precipitated resinous material. This material was filtered off and the aqueous solution evaporated to 20 ml. Ten ml. of 15 per cent sodium hydroxide solution were added, and the alkaline solution was shaken with 110 ml. of ether for 15 minutes, 5 ml. of a 2 per cent solution

of gum tragacanth being added prior to shaking. The ether extract was divided into equal parts and treated with a saturated solution of picrolonic acid in ether until precipitation was complete. The precipitate of alkaloid picronate was allowed to stand for 12 hours, after which it was collected on a tared Gooch crucible, washed with ether to remove picrolonic acid, dried at 105°C., and weighed. The weight obtained, multiplied by 0.561, gives the equivalent amount of berberine. The accuracy of the method was tested by applying it to a tissue of known alkaloidal value plus a known quantity of the purified alkaloid isolated from *M. trifoliolata*.

For determining the distribution of berberine in root tissues, sections 30 μ –40 μ in thickness were cut from the fresh roots by means of a sliding microtome and placed immediately in the various reagents recommended by Molisch (1923) for detecting berberine *in situ*. The most consistent results were obtained when the sections were treated with 2 per cent hydrochloric acid or with 2 per cent nitric acid. Bauer's (1908) method of forming berberine-acetone crystals also gave excellent results with this material. The procedure is as follows: fresh sections are floated in a few drops of water on a microscope slide, allowed to stand for a few seconds, warmed with one or two drops of sodium hydroxide solution (10 per cent), and treated with four or five drops of acetone. The sections are then covered and examined. For purposes of comparison, a few sections were placed in 5

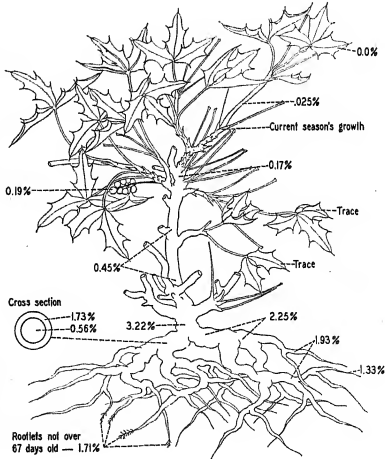


FIG. 1. Diagram of *Mahonia trifoliolata* showing concentration of berberine (dry weight basis) found in various parts of the plant. Drawing by Matilde Otero Watkins.

² The writers are indebted to Dr. S. O. Brown, who collected material from Brown County, and to Dr. C. H. Rogers, who furnished nursery plants from Temple.

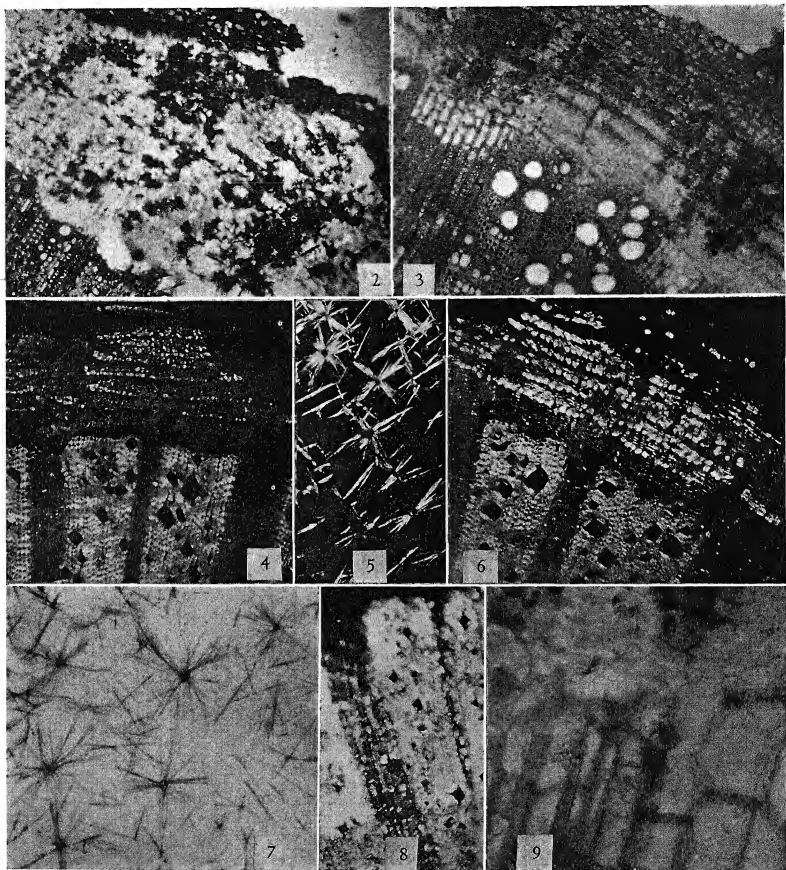


Fig. 2-9. Photographs showing distribution of berberine in roots of *Mahonia trifoliolata* and *M. Swaseyi*.—Fig. 2. Portion of transverse section of root of *M. Swaseyi*, showing irregular zones of berberine hydrochloride in the extra-cambial tissues. Unpolarized light. $\times 80$.—Fig. 3. A similar view for *M. trifoliolata*, showing zone of berberine-containing cells arching around the active phloem. Unpolarized light. $\times 80$.—Fig. 4. Portion of transverse section of root of *M. Swaseyi*, showing berberine (amorphous) in walls of xylem elements and in bast fibers. Polarized light. $\times 80$.—Fig. 5. Large crystals of berberine hydrochloride obtained by slow crystallization from solution. Polarized light. $\times 80$.—Fig. 6. Portion of transverse section of root of *M. trifoliolata*, showing berberine (amorphous) in walls of xylem elements and in bast fibers. Polarized light. $\times 80$.—Fig. 7. Same as Fig. 5. Unpolarized light. $\times 80$.—Fig. 8. Portion of stele of *M. trifoliolata*, showing crystals of berberine-acetone in vessels and tracheids and in the rays. Polarized light. $\times 80$.—Fig. 9. Portion of cambium in *M. trifoliolata*, showing young cells of a xylem ray beneath. Scattered aggregates of berberine hydrochloride crystals are seen. Unpolarized light. $\times 345$.

per cent tartaric acid in ethyl alcohol, shaken frequently, and after 24 hours removed, washed, and tested for alkaloids.

In studying the distribution of berberine in root tissues, the treated sections were observed microscopically with polarized and unpolarized light. All sections were examined critically in an attempt to distinguish in every case between crystals in true position and those which might have resulted from the dislocation of berberine by the knife.

The effect of berberine on the growth of *P. omnivorum* was tested by growing the fungus on a liquid medium to which the alkaloid had been added in various concentrations from 40 p.p.m. to 200 p.p.m. The nutrient solution used was a modification of Solution 70 of Ezekiel, Taubenhaus, and Fudge (1934) and consisted of MgSO_4 , 0.75 gm.; K_2HPO_4 , 1.35 gm.; NH_4NO_3 , 1.00 gm.; KCl, 0.15 gm.; and dextrose, 40.00 gm. per liter. After purification for heavy metals by the method of Steinberg (1935), the following were added: Mn, Cu, Fe, and Zn, each at a concentration of 2.5 p.p.m. (cf. Blank, 1938). The pure alkaloid was weighed into sterile plugged 250 ml. Florence flasks. To each flask was added one ml. of 95 per cent ethyl alcohol. The flasks were heated to 50°C. and placed aside until the alcohol evaporated. To each of the alkaloid and the control flasks were added 25 ml. of sterile nutrient solution. This technique was compared with that of autoclaving the alkaloid and the nutrient solution separately and then pouring the nutrient solution into the flask containing the alkaloid. Both techniques of sterilization gave similar results. In no case were the alkaloid and the nutrient solution heated while in contact with each other. After inoculation with the fungus, the cultures were incubated for three weeks at 28°C., after which the fungous mats were removed, dried at 80°C. under 3 to 4 cm. pressure, and weighed.

CHEMICAL RESULTS.—The general procedure for the extraction of alkaloids, when applied to roots of *M. trifoliolata*, yielded principally an alkaloid having the physical properties and chemical composition of berberine. Physical properties: melting point, crystallized from ether, 143°C. to 146°C., crystallized from chloroform, 179°C. (all melting points corrected); insoluble in ether, slightly soluble in chloroform and benzene, soluble in hot water and alcohol; neutral or very slightly basic to litmus. Chemical composition: calculated for berberine, $\text{C}_{20}\text{H}_{17}\text{NO}_4 \cdot 2\frac{1}{2}\text{H}_2\text{O}$; C, 63.13 per cent, H, 5.83 per cent, N, 3.68 per cent; found^a: C, 63.32 per cent, H, 5.60 per cent, N, 3.56 per cent.

Gravimetric determinations (method of Richter, 1914) of berberine in various organs of *M. trifoliolata* showed that the concentration of the alkaloid ranged from 1.33 per cent on the dry weight basis in some of the smaller lateral roots to 2.25 per cent in the largest roots. At the crown there was 3.22 per cent of berberine, which was the highest concentration

found in the entire plant. The percentage of berberine in the aerial parts ranged from 0.45 per cent in the older stems to none in the youngest leaves. The young roots formed after transplanting were found to contain 1.71 per cent berberine. Details of the concentration of the alkaloid in various parts of *M. trifoliolata* are shown diagrammatically in figure 1. For *M. Swaseyi* quantitative determinations were made only on the roots, in which the concentration of berberine was found to vary from 2.15 per cent to 2.48 per cent on the dry weight basis.

HISTOLOGICAL OBSERVATIONS.—The distribution of berberine in root tissues of *M. trifoliolata* and *M. Swaseyi* was studied in sections cut chiefly from roots 0.5 cm. to 1.0 cm. in diameter, in which considerable secondary thickening had occurred, and which showed a well developed periderm. For *M. trifoliolata* this account includes also observations on young roots containing only primary tissues.

As is well known, sections of berberine-containing organs, when treated with alkaloid reagents, do not show all of the berberine in crystalline form. A portion of the alkaloid, especially that impregnated in the thickened cell walls of bast fibers, tracheids, and vessels, usually remains amorphous. In the present work the sections treated with 2 per cent hydrochloric acid showed abundant crystallization of berberine hydrochloride in certain cells of the older phloem parenchyma. These extremely slender needle-shaped crystals are characteristically golden-yellow with a slight cast of green as seen in unpolarized light. They may occur singly or in small clusters, but more frequently are oriented as needles radiating in each case from a common center to form a dense rosette (fig. 2, 3, 5, 7, 10). Complete, more or less spherical rosettes are commonly seen occupying most of the cavities of the respective cells in which they were formed. Half-rosettes are formed in many cases along cell walls (fig. 10). Crystals of berberine nitrate formed in sections treated with 2 per cent nitric acid are similar to the hydrochloride in shape and arrangement. Sections treated with sodium hydroxide and acetone showed the characteristic reddish-yellow tablets of berberine-acetone crystals.

The distribution of berberine is in general similar in *M. trifoliolata* and *M. Swaseyi*. In both species the alkaloid is found impregnated in the walls of vessels and tracheids (fig. 4, 6, 8). With very few exceptions it is not seen in cell walls in the xylem rays. That the alkaloid is present in comparatively small amounts in cells of the rays is shown by the scattered occurrence of berberine-acetone crystals in this tissue following the sodium hydroxide-acetone treatment (fig. 8). The younger cells of the xylem rays are almost devoid of starch and frequently show crystals of berberine salts (fig. 9). The cambium and immediately adjacent cells as a rule do not appear to contain the alkaloid in abundance; occasionally small rosettes of crystals are formed in cambium cells opposite the rays (fig. 10).

^a Averages of duplicate determinations made by the Arlington Laboratories, Arlington, Va.

Outside the cambium in *M. trifoliolata* berberine usually occurs abundantly in a zone extending in arches from the cambium through the phloem rays and around the outer portions of the several strands of active phloem (fig. 3), which tissue generally contains less of the alkaloid. Beyond the active phloem the successive tangential layers of bast fibers are found to be uniformly yellow in unpolarized and polarized light and doubly refractive in the latter (fig. 3, 6). Occasional small rosettes of berberine salts may be found within the narrow lumina of these fibers (fig. 10). It seems very probable that the walls of the bast fibers are impregnated with berberine.

The distribution of the alkaloid is scattered in the periderm and, with the exception of occasional cells in which crystalline rosettes can be observed, seems to be confined to the layers of bast fibers which often occur in this tissue (fig. 10).

With respect to the distribution of berberine in cells outside of the cambium, *M. Swaseyi* differs from the above account only in the greater irregularity of the localization pattern as seen in transverse sections. The arches of berberine-containing cells around the active phloem are not so distinct. Groups of parenchymatous cells here and there throughout the tissues outside the stele appear to be packed with rosettes of crystals (fig. 2, 4). The bast fibers are berberine-impregnated (fig. 3), as in *M. trifoliolata*.

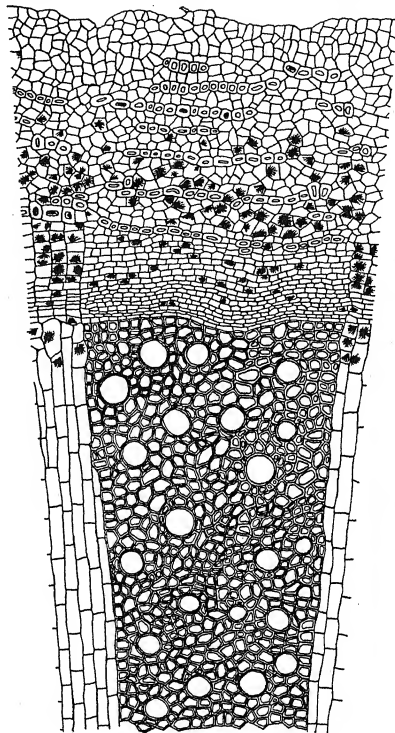
Sections of young roots of *M. trifoliolata* in which no secondary thickening had occurred showed crystals of berberine salts only in the pericycle. The cell walls of the endodermis, cortex, and epidermis, when examined with polarized light, appear yellow and doubly refractive, which suggests that berberine is evenly distributed in relatively low concentration throughout these tissues. The alkaloid occurs likewise in the walls of the various xylem constituents.

Fresh sections which had been treated first with 5 per cent tartaric acid in ethyl alcohol for 24 hours and subsequently placed in alkaloid reagents showed no berberine when examined microscopically.

RESULTS OF PURE CULTURE EXPERIMENTS.—The effect of berberine on the growth of *P. omnivorum* in pure culture was shown in experiments in which modified Solution 70 was used as a substratum, as described above. In this series of tests fungous mats of the following average weights were yielded in cultures in which the only variable was the amount of berberine: no berberine, 361 mg.; 40 p.p.m., 286 mg.; 100 p.p.m., 46 mg.; 200 p.p.m., no growth. Each of these weights is the average obtained from six cultures inoculated in pairs at three successive times.

DISCUSSION.—The function of alkaloids in the plant is still a subject of speculation. They are generally regarded as by-products of plant metabolism, in contrast to the simple bases and betaines that possibly constitute the building units for the formation of plant proteins. Other theories that have been advanced conceive alkaloids to be reserve materials involved in protein synthesis, plant stimulants or regu-

lators similar to hormones, or detoxication products rendered harmless to the plant by methylation, condensation, ring closure, and other synthetic processes (Small, 1938). Previous suggestions of the possible rôle of alkaloids in the resistance of certain plants to disease have been summarized by Greathouse (1938).



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Fig. 10. Semi-diagrammatic drawing of portion of transverse section of root of *M. trifoliolata*, showing typical distribution of berberine hydrochloride. $\times 127$.

The physical properties and chemical composition of the alkaloid isolated from *M. trifoliolata* and *M. Swaseyi* identify it as berberine. Examination of the data on the distribution of the alkaloid throughout the various organs and tissues reveals the fact that berberine occurs in highest concentration in the roots and especially in the bark of these organs. Older

main root tissues possessed a higher concentration of the alkaloid than the younger lateral roots. Young roots, not over 67 days old, formed after the bushes were transplanted contained 1.71 per cent berberine (fig. 1). Young actively growing stems formed in the current season contained very low concentrations (0.025 per cent) of the alkaloid. With increase in age nearer the base of the plant the alkaloid content of the stem increased. In no case was sufficient berberine present in the leaves to permit an accurate chemical estimation by the Richter method. Extracts of young leaves of the current season did not give any precipitate, whereas the intermediate and older leaves yielded from 0.2 to 0.4 mg. precipitate of berberine per 5 gms. of dried tissue, which quantity is designated here as a trace (fig. 1). A composite sample of the dried fruit yielded 0.19 per cent berberine.

A number of criteria were used in the microchemical determination of the localization of berberine in root tissues, as follows: the formation of characteristic aggregates of crystals of berberine salts following treatment of fresh sections with weak solutions of inorganic acids, the formation of characteristic berberine-acetone crystals after the sodium hydroxide-acetone treatment, double refraction in polarized light, and the failure of sections previously treated with alcoholic tartaric acid to show berberine salts when treated with weak solutions of inorganic acids. Present observations indicate that berberine, which occurs in roots of *Mahonia trifoliolata* and *M. Swaseyi* in at least sixty-five times the concentration shown experimentally to prevent growth of *P. omnivorum*, is widely distributed in the root tissues. Especially significant is the fact that in all roots examined a more or less continuous zone of berberine-containing parenchymatous cells was found just beneath the periderm. In both species then it would seem that there is sufficient berberine in the outer portions of the roots to inhibit or prevent invasive

growth of those hyphae of *P. omnivorum* which might come in contact with the periderm. The evidence suggests that the alkaloid, berberine, may be an important part of the mechanism of resistance in *M. trifoliolata* and *M. Swaseyi*.

SUMMARY

The alkaloid, berberine, is shown to be present in roots of *Mahonia trifoliolata* in concentrations varying from 1.33 to 2.25 per cent and in roots of *M. Swaseyi* in concentrations ranging from 2.15 to 2.48 per cent on the dry weight basis. The lowest of these concentrations is more than sixty-five times that shown experimentally to prevent completely the growth of *Phymatotrichum omnivorum*. The concentration of berberine is lower in the above-ground parts of the plant, ranging from 0.45 per cent in the older stems to none in the young leaves.

Sections of fresh roots of these species were treated microchemically to crystallize berberine in situ, and the distribution of the alkaloid in the various tissues was studied microscopically. Berberine was found widely distributed in walls of tracheids and vessels in the xylem and in smaller amounts in lumina of cells in the wood rays. In extra-cambial tissues the alkaloid occurs in a more or less continuous zone surrounding the active phloem. The bast fibers are impregnated with berberine, and small amounts are frequently observed in the periderm.

The generally continuous zone of berberine-containing parenchymatous cells, which surrounds the root just beneath the periderm, is considered to have especial significance as a possible factor in the resistance of *M. trifoliolata* and *M. Swaseyi* to *Phymatotrichum* root rot.

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EFFECTS OF AUXIN ON RATES, PERIODICITY, AND OSMOTIC RELATIONS IN EXUDATION¹

F. Skoog, T. C. Broyer, and K. A. Grossenbacher

IN EXPERIMENTS in which indole-3-acetic acid was applied to plants which were subsequently decapitated it was observed that bleeding was increased in treated plants. The process of exudation by which water and nutrients move through decapitated plants is not in all respects identical with the process of translocation in intact plants, since transpiration and perhaps other influences of the shoot have been eliminated or greatly reduced. Early experimenters conceived of transpiration and exudation as two forms of one physiological process. This view has in general been accepted and extended in so far as the processes which are responsible for exudation are now also considered to be active in a similar manner, though not directly measurable, in transpiring intact plants. An influence of auxin on exudation, therefore, is of interest in its possible relation to absorption and translocation of water and nutrients in the growth of plants.

In this article results of exudation experiments with *Pisum* and *Helianthus* are presented. The influences of applied indole-3-acetic acid on rates of bleeding, composition of exudates, and the characteristics of diurnal changes exhibited by the latter species are reported.

The experiments were carried out from October, 1936, to October, 1937.

EXPERIMENTS WITH PISUM.—*Material and methods.*—*Pisum* plants (Alaska 89) were grown in sand kept at suitable moisture content with tap water, or a solution² of similar known composition, in a dark room, at 24 to 26 degrees C. A low intensity ruby red light was used during observations. Eight to ten days after germination, when the plants had reached lengths of from 20 to 30 cm., they were decapitated, as specified, just below the upper or lower lateral bud (bud 2 and 1, respectively). Auxin paste, 10 milligrams of indole-3-acetic acid (Merck) dissolved directly in one gram of lanum anhydrous (Merck), was applied to the stems. Care was taken to treat all plants alike, each with about 5 mg. of paste. The same amounts of auxin-free lanolin paste were applied to an equal number of control plants grown in the same vessel. Glass tubes, tapered to fit, were carefully slipped about 3 to 5 mm. over the cut ends. Exudates collecting in these tubes were removed and measured at intervals.

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² This solution, based on analyses of tap water, was kindly supplied by Mr. A. Ulrich.

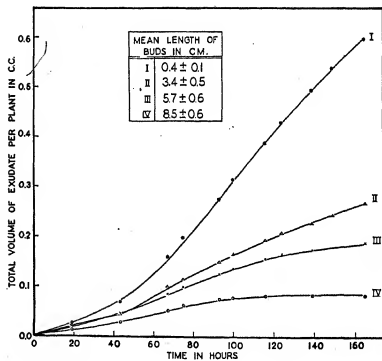
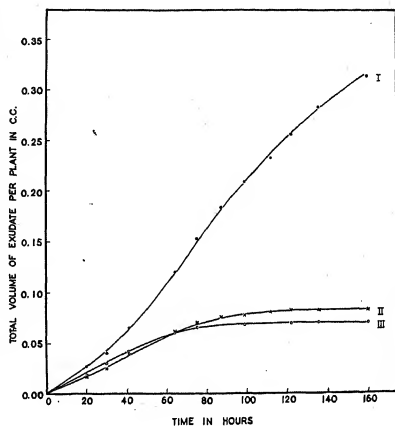


Fig. 1 (above). Effect of auxin on exudation in *Pisum*. Curve I treated with auxin in lanoline paste. Curve II lanolin paste without auxin. Curve III without paste. Test started 3/10/37.

Fig. 2 (below). Effect of concentration of auxin on exudation in *Pisum*.

Curve I II III IV
Concs. in mg./gm. of lanoline 10 2 0.4 0

Inset shows mean lengths of lateral buds. Test started 5/26/37.

In preliminary experiments it was found that the variability in bleeding from individual plants was such that mean values from sets of about 15 plants would show statistically significant effects of auxin treatment. In the experiments to be reported 36 to 50 plants were used for each treatment.

Influence of auxin on exudation.—In five successive experiments, one of which is shown in figure 1, plants were decapitated below bud 2. The paste was applied to the tips including the cut surfaces of the stumps. Comparison of curves I and II shows the marked increase both in rate and in duration of exudation resulting from the application of auxin. The close agreement between curves II and III, which represent exudation from plants with and without auxin-free lanoline paste, shows that under the conditions of our experiments no constituent of the lanoline paste affects bleeding.

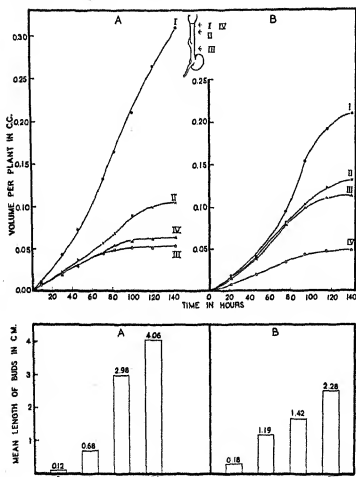


Fig. 3A and B. Effect of position of application of auxin to the stems on exudation and bud development in *Pisum*. Positions of treatment designated in the diagram. Curves and columns I, II, and III with auxin paste, IV plain lanoline paste without auxin. Tests started 5/13/37 and 4/29/37, respectively.

Effect of concentration of auxin on exudation.—

Results from several experiments, one of which is represented in figure 2, show that the increase in exudation is a function of the concentration of auxin applied. These concentrations were in all cases relatively high, but since the entry of auxin from anhydrous lanoline paste is slow (a single application is not completely utilized in ten days), the active concentration within the cells might not be markedly

greater at any given time than that normally existing in plants with intact terminal buds. The amounts of auxin used were not sufficient to exert any visible toxic effects nor to cause bleeding by an increase in osmotic pressure. Even if all auxin added were immediately absorbed either into the stumps or into the roots, the osmotic concentration of the tissues would be increased only by a fraction of one per cent of that of electrolytes present, as determined by conductivity measurements of expressed sap. The effect of auxin in exudation is therefore different from the osmotic action commonly associated with absorbed electrolytes (Hoagland and Broyer, 1936b), as will also be demonstrated later.

Relation of bud growth to exudation.—It has been shown (Thimann and Skoog, 1934; Skoog and Thimann, 1934) that in decapitated auxin treated plants the outgrowth of lateral buds is prevented. In the present experiments it was noticed that stimulation of exudation and inhibition of bud growth occurred together and as functions of the concentrations of auxin applied. As an example of this correlation, the final mean lengths of the lateral buds of plants whose exudation is plotted in figure 2 are shown in the inset of the figure.

Effectiveness of auxin with respect to the place of application to the stem.—a. In inhibition of bud growth.—In an attempt to separate the effect of auxin in exudation from that in bud inhibition, auxin was applied to different positions on the stems. It is known (Skoog, 1936) that the effectiveness of auxin in bud inhibition depends on the place of application to the stem with respect to the position of the lateral buds. The effectiveness, however, in the case of indole-3-acetic acid is not greatly dependent on the length of stem between the point of application and lateral buds situated below this point (Thimann, 1935). A single application of auxin, in the stated concentration, at the tip, covering also the cut surface of the stump, caused practically complete bud inhibition. Auxin applied to the stump just below the cut surface was slightly less effective. Application between buds 1 and 2 caused marked inhibition only of bud 1. Application below bud 1 had no consistent effect on lateral bud growth, although an occasional slight inhibition and in other cases a significant but minor stimulation of bud growth have been observed. The mean bud lengths obtained in one typical experiment were for the four different auxin treatments 0.39, 2.57, 5.03, and 7.88 cm. respectively as compared with 7.51 cm. for control lanoline treatments and 0.24 cm. for intact control plants. These results should be distinguished from those obtained by basal application of aqueous auxin solutions, from which auxin is transported upward through the xylem into the stem and thus may exert the same effects as though applied from above (Skoog, 1938).^a

b. *In exudation.*—The effect of auxin in exudation was compared with that in bud inhibition. The treatments were comparable, except that the plants were

^a Compare Le Faut, 1936; and Snow, 1938.

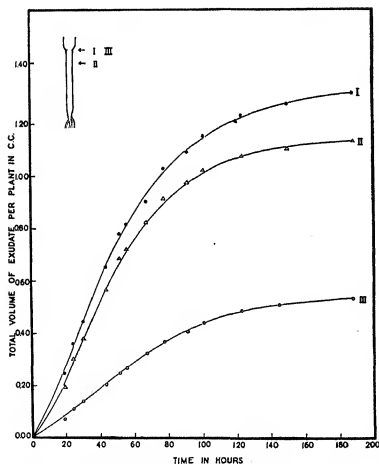
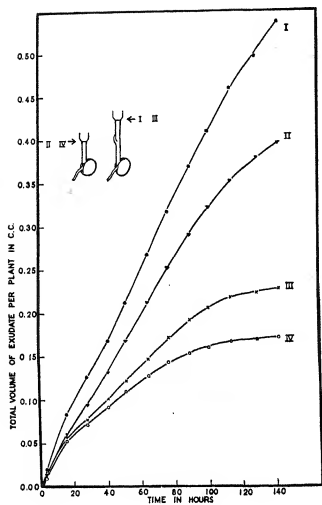


Fig. 4 (above). Comparison of the effect of auxin on exudation in *Pisum* of different stem lengths. Curves I and II represent long and short plants respectively with auxin. Curves III and IV long and short controls. Test started 2/20/37.

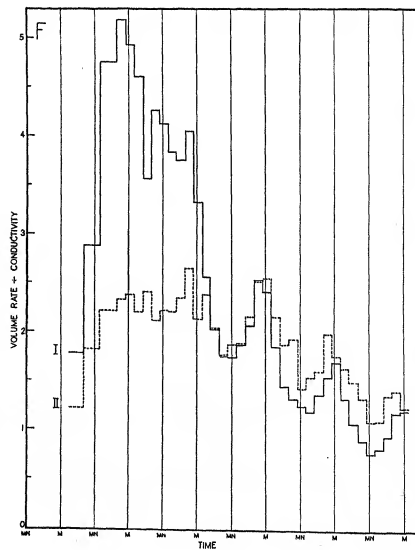
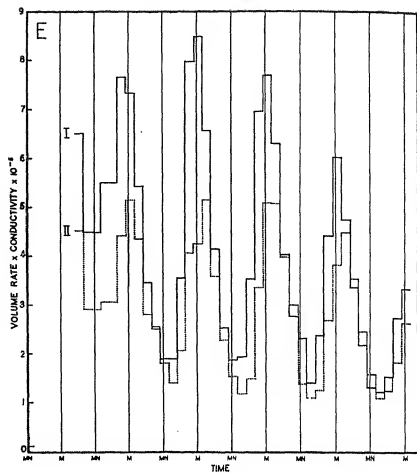
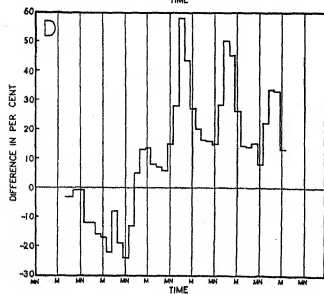
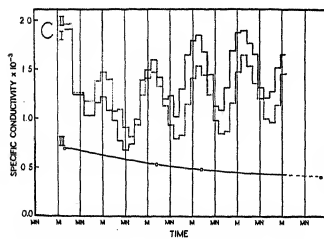
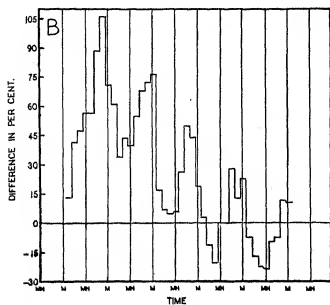
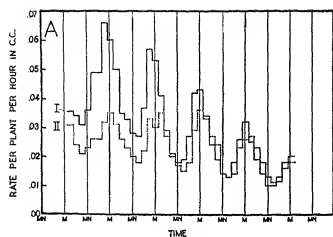
Fig. 5 (below). Effect of auxin on volumes of exudate

decapitated below bud 2. In five successive experiments, including the two shown in figures 3A and 3B, the following results were obtained: In all experiments auxin applied to the tips of the stumps was most effective in increasing exudation, and application below the cut surfaces was less effective. In four experiments application below bud 1 was completely ineffective; however, in one experiment (fig. 3B, Curve III) it caused a significant increase in exudation and also some bud inhibition. It is seen from the figures that parallel effects are obtained on exudation and bud inhibition.

The ineffectiveness of auxin applied below bud 1 might be due to a lower sensitivity of these more mature tissues of the stem. Since the influence of position of application is of importance, this point was further studied by experiments with plants of different stem lengths. Plants were divided into two equal groups, those of the first were decapitated below bud 2 (long plants) and those of the second below bud 1 (short plants). Auxin paste was applied to half the number of plants in each group. In one experiment (fig. 4) the long plants treated with auxin bled in total volume 30 per cent more than the treated short plants. However, a similar difference, 28 per cent, existed between long and short control plants. The effect of auxin was therefore the same in the two sets of plants. In a second experiment long and short auxin treated plants bled in total volume 430 and 605 per cent more than their respective controls. The exudate volumes in the control sets were within 2.5 per cent of each other. In this experiment the short plants actually bled 17 per cent more in response to the applied auxin. These results were likewise obtained with plants of the same age and appearance, but not, however, all growing in the same vessel. It appears certain, therefore, that the application of auxin to the lower regions of the stems may be equally effective in promoting bleeding as that to more apical regions provided that the superimposed influence of lateral buds situated above the point of application is removed.

The results demonstrate that auxin applied to the stems of decapitated *Pisum* plants may stimulate exudation. The conditions under which auxin is effective and the magnitude of the effect parallel the conditions and the magnitude of the effect of auxin in bud inhibition. A separation of the effect of auxin in the two processes was not possible in *Pisum*, because even in plants without other lateral buds, the cotyledonary buds developed in control plants. The difference in exudate volumes between treated and control plants does not represent merely a difference in amounts of solution utilized in growth, because in *Helianthus* and *Zea*, on which no buds developed, marked increases in exudation have also been obtained by auxin treatment. Nevertheless, the magnitude of the increase in exudation may be due in

from *Helianthus*. The positions of application of auxin are as designated in the inset. Curves I and II with auxin, Curve III control. Test started 5/20/37.



part to the retardation of bud growth. Whether, on the contrary, the effect of auxin in bud inhibition may be ascribed to its effect in exudation, which would be in essential agreement with the mechanisms of correlative inhibition proposed by Moreland (1934) and others, cannot be decided from the present results.

EXPERIMENTS WITH *HELIANTHUS*.—*Material and methods.*—*Helianthus annuus* plants (Russian Mammoth) were grown in a greenhouse in 4-litre tanks, each containing 96 seedlings and supplied with a complete culture solution (Hoagland and Snyder, 1933⁴). At the same time when two to three pairs of leaves were well developed (approximate age four weeks) the plants were moved into the dark room. In experiments of the types shown in figs. 5 and 6, the roots were rinsed with distilled water and thereafter kept in continuously aerated 0.005 molar KCl solutions. In those represented by fig. 7, they were allowed to remain, without washing, in the culture solutions in which they had been grown. The plants were decapitated below the cotyledons. The lanoline pastes were applied and exudates were collected from the hypocotyls by methods similar to those used for experiments with *Pisum*.

Effect of auxin on exudation.—From the curves of figure 5, representing total volumes of exudate per plant plotted against time, it is seen that in *Helianthus* also auxin markedly increases the rate of exudation. In a second experiment, application at the tips including the cut surfaces was, as with *Pisum*, relatively more effective than that only below the cut surfaces. Therefore, the former treatment was used, except in experiments where total solids in the exudates were to be determined.

A striking difference between the types of response to auxin given by *Pisum* and *Helianthus* may be observed. In the former, auxin caused an increase both in rate and in duration of exudation. In the latter, only an increase in rate was obtained, which reached a maximum one or two days after application and then gradually decreased on successive days.

A bleeding response to auxin in *Helianthus* is limited to plants grown under certain conditions. In several experiments with young seedlings grown in the dark room or with older plants grown in low intensity light, the plants bled for only one to two days and at a rapidly decreasing rate. In some of these experiments a slight stimulation was perhaps produced by auxin, while in others a definite retardation of exudation was observed. *Zea* seedlings, however, decapitated below the coleoptile node and treated with auxin, like *Pisum*, bled for more than a

⁴Standard Hoagland's solution plus supplementary solutions A and B.

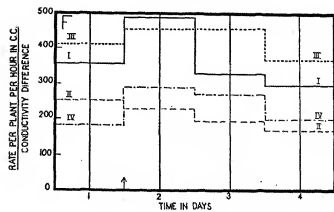
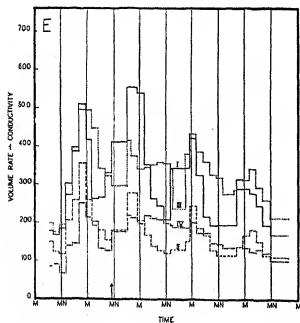
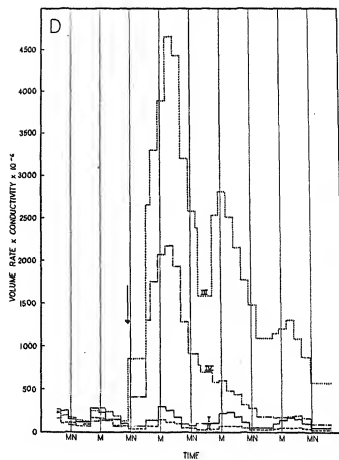
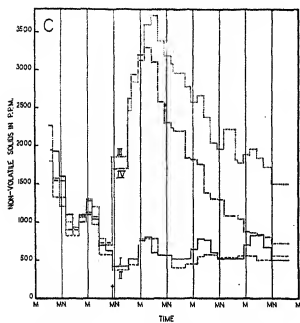
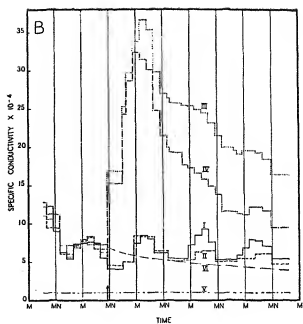
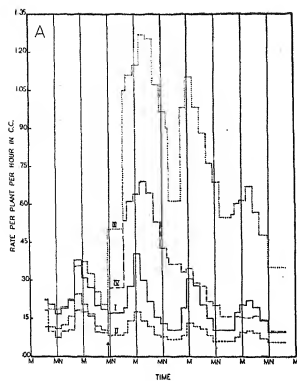
week at rates greater than those of the controls. In *Zea*, as in *Pisum*, the seeds are retained after decapitation. The fact that the response in the three kinds of seedlings depends upon the presence of seeds or, in the absence of seeds, depends on previous light conditions indicates that the stored material is required for continued exudation. It furthermore suggests that the influence of auxin is related to the utilization of this storage material. This interpretation may also be supported by a consideration of the rates of bleeding in terms of composition of exudates.

Diurnal periodicity in exudation.—In agreement with work on root pressure variations (Grossenbacher, 1938b), a marked diurnal periodicity in exudation was observed in *Helianthus*. Hofmeister (1858, 1862) has reported that diurnal fluctuations can occur independently of changes in the environment during the time of observations, but may of course be modified by such changes. This observation was confirmed by Baranetzky (1877) on *Helianthus*, *Ricinus*, and other plants. However, he related the diurnal periodicity in exudation to changes in external factors existent during the growth period previous to experimentation, particularly to light. A relationship to changes in illumination was clearly indicated from his experiments, especially with *Helianthus tuberosus*, in which daylight was restricted during the growing period to certain portions of the normal day with consequent changes in the times of appearance of maximum and minimum rates of exudation. Baranetzky also confirmed the earlier observations by Brücke that the magnitude of the diurnal fluctuations in exudation increases with the age of the plant up to the time of flowering and then rapidly decreases. Thus young plants may exhibit a regular diurnal cycle for one or two days and may then vary irregularly, whereas older plants maintain the periodicity for a longer time (in our experiments at least five days). Heyl (1933) on the contrary ascribes diurnal changes in rate of exudation observed in *Brassica* to concurrent fluctuations in temperature.

In the present experiments the existence of diurnal fluctuations in exudations in *Helianthus* was ascertained in environmentally controlled dark rooms at Berkeley and later at Harvard. In the latter location the dark room was maintained at 24 degrees C. and at about 80 per cent relative humidity by means of an air circulation system. The variations during a 24-hour cycle were within ± 0.5 degrees and ± 0.5 per cent, respectively. The observed periodicity in exudation bore no relationship to the registered variations within this range.

In the experiments with *Pisum* seedlings no periodicity in the rates of exudation was observed. The slower rates of bleeding of these plants might have

Fig. 6. Comparison of effect of auxin on rates of exudation and composition of the exudates in *Helianthus*. Curves I auxin treated, Curves II controls. Time periods marked M, are noon; MN, midnight. Kept in 0.005 M. KCl solution. A, volume rates of exudation; B, percentage difference in rates of exudation between auxin treated and control plants; C, specific conductivities of exudates and the external solution (Curve III); D, percentage difference in conductivities of exudates from auxin treated and control plants; E, rates of electrolyte exudation; F, rates of exudation per unit electrolyte in exudates. Test started 7/21/37.



prevented its detection, but if any periodicity of exudation be present in *Pisum* grown in the dark, its magnitude is not comparable with that in *Helianthus* previously grown in the light.

In experiments with *Pisum* previously grown in the light and with *Helianthus* seedlings grown in the dark, the rates of bleeding were inadequate for reliable measurements of diurnal changes in rate of exudation. Whether or not the diurnal periodicity was absent in seedlings grown in the dark, as suggested by Baranetzky, is therefore not clearly indicated. His observation that previous illumination may be a controlling factor in the periodicity, however, has been confirmed and extended in experiments with *Helianthus* exposed to supplementary light from a carbon arc at definite daily intervals during the growing period (Skog, unpublished) and in experiments carried out in environmentally controlled cabinets from the time of germination of the seeds (Grossenbacher, 1938a). From the latter data, in which the periodicity has been controlled, it may be concluded that the diurnal periodicity in the present experiments has become associated with the plants prior to the starting of exudation and that it is maintained independently of external changes during the time of measurements.

The volumes of exudates were measured at three-hour intervals, and the data are expressed as c.c. per plant per hour. Results from one experiment, which are typical of several others, are presented in fig. 6. As shown in part A, there are distinct periods of rapid and slow rates of exudation in all plants during each 24-hour interval. The maxima and minima correspond roughly to noon and midnight, respectively, in all experiments to be reported. The rates of bleeding decrease on successive days following the first or second. It should be noticed, however, that the magnitudes of the diurnal fluctuations are greater than the decrease from any given period to the corresponding one on successive days.

Diurnal periodicity in effectiveness of auxin.—The influence of auxin is shown clearly in fig. 6B, where the differences in bleeding rates per hour between treated and controlled plants, expressed as percentage of the controls, are plotted against time. In the early periods after application auxin produces a marked acceleration throughout the 24-hour cycle. During the later periods, however, the maximum effectiveness is in the high, especially rising portions of the curves; in the low portions auxin may produce little or no effect.

From the cyclic effectiveness of auxin in exudation it is concluded that its chief influence is not a result of any increase in size which may occur either in

stems or roots of auxin treated plants. This conclusion has been substantiated by experiments showing that exudation rates per unit final fresh weight of the tissues were greater in auxin treated plants.⁵

EFFECT OF AUXIN ON COMPOSITION OF EXUDATES.—Several investigators have proposed that the rate of water movement (rate of exudation) into and through plants is regulated by the osmotic relations of the root system (Blackman, 1921; Sabinin, 1925; Scott and Priestley, 1928; Crafts and Broyer, 1938). It is unlikely that applied auxin exerts any appreciable direct osmotic effect. It is possible, however, that it indirectly affects the osmotic concentrations of the plant either through an influence on the absorption of salts or on the liberation of osmotically active material within the cells of roots or shoots. These possibilities will be considered in turn.

Determinations of electrolytes.—a. In *Pisum*.—Several experiments were conducted with *Pisum* in which the concentrations of electrolytes present in the stem stumps and in roots of control and auxin-treated plants were determined by conductivity measurements of sap expressed from frozen tissues (Hoagland and Broyer, 1936a). Slightly higher concentrations were frequently present in roots from treated plants, but when the concentration of auxin was varied, the differences in conductivity were not always consistent with the corresponding differences in amounts of exudates. Any relationship between amount of bleeding and concentration of electrolytes which may exist in the roots would probably have been obscured by the unequal bud development in control and treated plants. Reliable electrolyte concentration measurements could not be made on the exudates because the rates of bleeding were too slow to afford volumes suitable for analysis.

b. In *Helianthus*.—Since the effect of auxin on exudation in *Helianthus* is transient and fluctuates diurnally, conductivity measurements of expressed sap were made at the end of a few experiments only, but the electrolyte contents of exudates were satisfactorily measured. As an example, the results from the plants of figure 6 are included in part C of the figure. Curves I, II and III represent specific conductivities of exudates from auxin treated and control plants and of the external solutions, respectively. The specific conductivities of the exudates are much higher than that of the external solution and are of the same order as were found in the expressed sap (compare Hoagland and Broyer, 1936b; Sabinin, 1925). They fluctuate diurnally roughly parallel with the rates of

⁵ The possibility that the periodicity in exudation may be related to diurnal cycles in growth rates has not been studied.

Fig. 7. Relationships between the effects of auxin and nutrient salts in exudation of *Helianthus*. Curves I auxin treated, Curves II controls, both without additional nutrient salts. Curves III auxin treated, Curves IV controls both with additional nutrient salts supplied at arrow. Curves V and VI specific conductivities of culture solutions without and with additional nutrient salts respectively. A, volume rates of exudation; B, specific conductivities of exudates and external solutions (Curve V without additional salts; Curve VI with additional salts); C, concentrations of total non-volatile solids in exudates; D, rates of electrolyte exudation; E, rates of exudation per unit electrolyte in exudates; F, diagram showing average daily rates of exudation per unit electrolyte difference between exudates and external solutions. Test started 8/14/37.

bleeding, except that the maxima often occur slightly later. The conductivity measurements further show that in the early intervals after application of auxin, when the rate of bleeding is markedly higher in treated plants, the salt concentrations of exudates from treated plants rarely exceed and are frequently less than those from controls. During later periods, when the rates of bleeding are more nearly equal in the two sets, the salt concentrations from treated plants are higher. The rates of total salt exudation, expressed by specific conductivities \times volume rates, (fig. 6E) are thus consistently higher in treated plants and undergo more marked diurnal fluctuations. Nevertheless, the data do not reveal any simple relationship between the rates of salt exudation and auxin action in bleeding. Comparisons of figures 6A and B, and 6C and D show that with respect both to rates of exudation and concentrations of electrolytes in exudates the largest effect of auxin is exerted in the rising portions of the diurnal cycles, preceding the intervals of most rapid exudation. This suggests rather a relationship between auxin and the mechanism responsible for the diurnal fluctuations. Before considering further the respective roles of auxin and electrolytes in exudation, additional data will be presented from experiments in which plants were supplied with two different concentrations of salts.

Plants, kept in the partially depleted culture solution in which they had been grown, were divided into four sets, of which two were treated with auxin. The lanoline pastes were applied to the hypocotyls just below the collecting tubes to avoid lanoline contamination of the exudates as a source of error in the determinations of total solids. The rates of exudation were determined over several three-hour intervals, and then mineral salts (full strength Hoagland's solution) were supplied to one set each of control and treated plants. Volumes of exudates, their specific conductivities, and total non-volatile solid contents, obtained after evaporation in a ventilated oven at 75 degrees C., were measured. The data in figure 7 represent results from one of two experiments in which identical effects were observed.

As shown in parts A, B, and C of the figure, the introduction of additional salts was followed by rapid increases in bleeding rates, electrolyte, and total non-volatile solid contents of the exudates. The effect of auxin on the rates of exudation is of the same type as before (fig. 6) and is increased by additional salt. The rate of solute exudation (fig. 6D) is increased about ten times by salt application and about thirty times through the combined influences of applied auxin and salts. The stimulation reached a maximum ten to twenty hours after salt was added to the external solution.

An indication of the respective roles of concentration of salts and of auxin on the rates of bleeding may be gained from the ratios: rate of exudation \div concentration of electrolytes in exudate. These ratios computed for successive intervals for each set of plants are shown in part F of figure 6 and part E of

figure 7 and refer to these figures respectively. A comparable graph, figure 7F, which, however, covers twenty-four hour intervals and is based on concentration differences between the exudates and the external solutions, is included for the latter experiment. If the rate of bleeding were determined merely by the salt concentration in the xylem as in a simple osmotic system (see Crafts and Broyer, 1938), it might be expected that the values for the ratios from a given set of plants would be nearly constant throughout the experiment. This condition is approached in the control plants after application of a high concentration of salts (Curve IV, fig. 7E). In all other curves, on the contrary, the values vary diurnally for control as well as for auxin treated plants.

It is obvious from the shape of the curves that auxin does not act as an electrolyte. Furthermore, if auxin were effective merely through an influence on the salt concentration, the ratios for treated and control plants should be expected at least to possess a constant relation to each other. On the contrary, as for example in figure 6F, the ratios for treated plants rise to very high values in the early periods after auxin application, then gradually fall to approach those of the controls and often become even slightly lower during the latest intervals. It becomes apparent, especially from a comparison of the curves of figure 7E, that the effects of auxin and of salts on exudation are exerted mainly in separate mechanisms. The values of the ratios for the two sets with applied auxin are relatively higher than for the controls and exhibit a more marked diurnal periodicity both in the absence (Curve I) and presence (Curve III) of additional salts. Of the two, the latter curve is lower during the twelve hours immediately after salt addition and subsequently tends to be higher, especially in the intervals of decreasing exudation rates. The curves for the two sets without applied auxin are both low and are of similar shapes up to the time of salt addition. Thereafter, curve IV, which indicates the effect of additional salts, exhibits no diurnal periodicity. During the day it is lower and during the night it is higher than curve II. Thus, also, the differences between the ratios for corresponding treated and controlled plants vary both diurnally and with time from the beginning of the experiment. It follows that auxin does not act in exudation primarily by increasing the concentration of salts, even though it greatly accelerates the rate of movement of both salts and water through the plants and may lead to increases in salt concentration of the exudates.

It may be observed, however, that the rate of bleeding may increase with increasing concentrations of salts faster than the increase in concentration difference between exudates and the external solution. In auxin treated plants in which the concentrations of electrolytes increase, the rates of bleeding per unit concentration of salts will consequently also increase. Under these conditions, therefore, the influence of auxin may to a small extent be exerted through an

effect on salt concentrations. That this is not the main function of auxin in exudation, however, is obvious from a comparison of curves I and IV, figure 7F. The ratios expressed by the former curve are much higher in spite of their pertaining to lower concentrations of electrolytes. It is in fact possible to have increases in rate of exudation caused by auxin accompanied by either increases or decreases in salt concentrations of the exudates.

It has been indicated that the effect of auxin in exudation is closely related to the mechanism responsible for diurnal periodicity. This relationship is particularly evident in figure 7E. In the plants represented by curve IV, auxin apparently limits the rate of exudation during a portion of the day, because its presence, as in curve III, not only causes an increased exudation rate but also is sufficient to maintain large diurnal fluctuations, otherwise not apparent, in plants supplied with additional salts. The mechanism responsible for the diurnal periodicity may of course be operative in the control plants supplied with salts, to an extent comparable to that in the control plants without additional salts, but in the former its effect is obscured by the large salt effect. It should be noted in this connection that the control plants also contain some auxin at the start of the experiment (unpublished data; also compare Meyer, 1936), which supply is only gradually depleted (see Thimann and Skoog, 1934). It is likely that this auxin influences the rate of exudation and periodicity of the control plants. On this basis, applied indole-3-acetic acid may exert a quantitative effect, due to increased auxin concentration, rather than a qualitative effect on exudation. It is not inferred, however, that auxin itself is responsible for the diurnal periodicity, but merely that it plays a part in this complex phenomenon.

From the data presented above it is concluded that a consistent causal relationship between concentration of salts in the exudates and exudation rates (the former used as a measure of the concentration within the xylem) can obtain only under such conditions, as in curve IV, that the salt effect is predominant. Moreover, the variations observed under different treatments are such that the validity of this conclusion is not limited to the assumption of a strictly linear relationship between salt concentration and exudation rate.

The mentioned features of the graphs are characteristic of the different treatments used. They are individually and collectively consistent with the view that auxin acts in the mechanism responsible for the diurnal periodicity, and salts are effective mainly in a separate mechanism.

Determination of non-volatile solids in exudates.—Attempts have been made to detect the presence of non-electrolytes which might act osmotically in exudation. Determinations of non-volatile total solid contents, made on successive portions of exudates parallel with conductivity measurements, are shown in figure 7C. From comparisons of these curves with

the corresponding ones of figure 7B, it is clear that the concentrations of non-volatile total solids follow the concentrations of electrolytes very closely both for auxin treated and control plants. Hence, the rates of exudation per unit concentration of total solids are nearly the same as those for electrolytes in spite of the wide range covered by these ratios (1 to 5).

Since exudates from certain trees, during the season of rapid exudation, contain considerable amounts of sugar, tests were made for reducing sugars on a number of samples of *Helianthus* exudates. Appreciable amounts could not be detected. Presumably the exudates, nevertheless, contain small amounts of organic materials (Litvinov, 1927), and possibly there exists within the limits of error of the present determinations some significant variation between salt and total solid contents in the exudates. Even if non-electrolytes were present in sufficient concentration to exert an influence on exudation, it is evidently impossible, on the basis of composition of the exudates, to ascribe either the diurnal fluctuations, or the differences in exudation between auxin treated and control plants, to variations in non-electrolyte concentration. The present results suggest rather that non-electrolyte solutes play directly only a small, relatively constant, and possibly gradually decreasing role in the exudation process in the present experiments.

Determination of respiration.—One experiment has been made in an attempt to correlate the increase in exudation due to auxin treatment and the diurnal periodicity with changes in respiration. Measurements of carbon dioxide production per six-hour intervals were made by essentially the method reported by Hoagland and Broyer (1936a) on duplicate sets of decapitated auxin treated and control *Helianthus* plants. These were suspended so that only the roots were immersed in continuously aerated 0.005 molar KCl solutions. For the first six hours the respiration rate of roots and stumps combined was of a high value (0.34 mg. CO_2 /gm./hr.). It fell rapidly in the next 12 hours (to 0.08 mg./gm./hr.) and remained relatively constant up to 48 hours. During the next 24 hours the rate gradually rose, perhaps as a result of enhanced activity of microorganisms. No significant diurnal fluctuations nor changes due to auxin treatment were observed in respiration, but they were obtained with respect to exudation in an accompanying experiment with plants of the same group. From the results of this experiment, in agreement with those obtained under similar conditions by Grossenbacher (1938b) and also with earlier findings (see Romell, 1918), there is no correlation apparent between the changes in rates of exudation and total respiratory activity. The possibility remains that under more appropriate conditions such a correlation would be demonstrable.

Discussion.—It is realized that the effectiveness of the osmotic forces in exudation depends on the resistance of the tissues to the movement of water

and solutes. A partial explanation of periodicity and the influence of auxin in exudation in the present experiments may perhaps be found in terms of changes in permeability. There are difficulties, however, with this interpretation. Both the rate and pressure of exudation undergo parallel diurnal fluctuations. Changes in permeability to water or to solutes may affect the rate of pressure change but cannot by themselves be responsible for the recurring maximum and minimum pressures. An explanation of these as well as the effect of auxin in exudation must also involve changes in metabolic activity.

From the investigations of Steward (1937), Lundegårdh (1934), and Hoagland and Broyer (1936b) it is evident that both absorption of salts and exudation are related to respiration. These findings imply the need in continued exudation for a metabolite (presumably carbohydrate) and also suggest a plausible explanation for the relatively high requirement of oxygen (Hoagland, 1936; Crafts and Broyer, 1938). They do not, however, offer any clue to the cause of the diurnal periodicity nor completely explain the influence of applied auxin in exudation, unless it is assumed that there is a periodic liberation and removal within the plant of material which acts osmotically, and further, that auxin at least in part acts in conjunction with this material. Measurements of the composition of the exudates and of respiration of exuding plants did not indicate any such material. Indirect evidence, nevertheless, suggests that utilization of food is an essential requirement for continued exudation and that the effect of auxin is associated with this process. Similarly, experiments on the effect of auxin on growth have led to the conclusion that its action may be connected with respiration,² but attempts to demonstrate such a relationship manometrically have thus far failed (Bonner, 1933; van Hulssen, 1936; Yin, 1937). However, experiments by Thimann and Sweeney (1937, 1938) on the relation of auxin to protoplasmic streaming show that its influence in this process (generally an enhancement thereof) is limited by sugar and oxygen and can be affected by various agents which affect respiration. Experiments by Schneider (1938) further show that auxin acts in conjunction with sugar in promoting growth of the *Avena* coleoptile.

It is suggested that the amounts of auxin originally present in intact plants and the smaller quantities in decapitated plants may influence the absorption and translocation of water and salts. Plasmolysis studies on *Helianthus* by Ruge (1937) have likewise led to the conclusion that auxin promotes salt absorption. However, the separation of the effect of auxin into two stages, first on plasticity of the cell walls and only later on salt uptake, cannot be supported by our results, since the effect of auxin in exudation could often be measured three hours after application and may occur sooner, especially as it is impossible to distinguish whether auxin acts in the tissues of the stem or must penetrate to the roots.

² Attention is called to a recent article by Pratt (1938).

The present results demonstrate that applied auxin influences translocation of water and solutes and thus the osmotic relationships in plants. They suggest that auxin may be a regulating factor not only in growth and tropic movements but also in the relative distribution of materials within non-growing tissues (as for example in turgor movements). In this respect the results constitute further evidence in support of the "mobilization" activity of auxin, proposed by Went (1936), but only an effect on rate rather than on direction of movement has been studied.

SUMMARY

A stimulating effect of auxin, indole-3-acetic acid, on exudation has been studied principally in *Pisum* seedlings grown in the dark and *Helianthus* plants grown in culture solutions in the greenhouse, but kept in controlled dark rooms during the time of exudation.

In *Pisum*, auxin increases both the rate and duration of exudation. Its effect is modified by an accompanying influence on bud inhibition. Both phenomena are comparably affected with respect to concentration of auxin and to the position of its application to the stems. Nevertheless, the effect of auxin in exudation need not be dependent on its role in bud inhibition.

In *Helianthus* the stimulating effect of auxin is mainly on the rate of exudation. It promotes the exudation of both water and salts, but may lead to either increases or decreases in concentration of salts in the exudates.

The effect of auxin is not due to any increase in total growth of treated plants. It is not a simple osmotic effect, nor is it mainly directly connected with the mechanism whereby solutes are effective in exudation.

The rates of exudation and electrolyte concentrations of the exudates are greatly influenced by the salts supplied to the external solutions, but the ratios of rate of exudation to concentration difference between the exudates and the external solutions may vary over a wide range and are increased by the presence of auxin.

Indirect evidence suggests that auxin acts in conjunction with the utilization of food required for continued exudation, since a stimulating effect appears to be limited to plants rich in storage materials. However, non-electrolyte solutes appear to play only a minor role directly in exudation, and no correlation was obtained between auxin activity or diurnal cycles in exudation and total respiratory rates.

The effect of applied auxin is exerted in the mechanism responsible for the diurnal periodicity in exudation shown to be exhibited by *Helianthus*, and it is greatest during the periods of increasing bleeding rates.

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CONTRIBUTIONS TO THE CHEMISTRY OF THE PLANT CELL WALL.

VIII. THE CELLULOSE IN HAIR CAP MOSS¹

W. M. Harlow and L. E. Wise

THE QUESTION of whether there is one cellulose or many different ones within the cell walls of plants has received considerable attention among botanists and chemists. Some of the latter have felt that each plant species or group of species had its own cellulose which differed in chemical constitution from that of other plants. Others have thought that analytical differences could be explained on the basis of varying amounts of extraneous materials.

been included. The lower yield for the moss may indicate that another or other polysaccharides besides cellulose are present. This appears doubtful. It is more probable that it was not possible to duplicate exactly the very delicate analytical procedure. 2 gm. samples should be used in the acetolysis because of the difficulty of measuring with adequate precision the small quantities of reagents used. Because of the labor involved in dissecting sufficient setae, the deter-

	Percentage of of cellulose after ethanolamine extraction of raw material	Percentage of alpha cellulose in the preceding	Percentage of theoretical yield of cellobiose octa- acetate (crude)	Melting point of purified cellobiose octaacetate
Moss	32.4	61.0	35.0	227.5°C.
Fern	30.0	57.0	43.4	228.0°C.
Cotton	—	—	40.0	
			41.5	227.5°C.
Melting point of a 50-50 mixture of octaacetate from moss and cotton, respectively				227.5°C.

One of the best chemical tests for cellulose, as represented by cotton, depends on its conversion into cellobiose octaacetate under carefully controlled conditions of time, temperature, and concentration of reagents (Spencer, 1929). The formation of this substance indicates a repeated anhydrocellobiose linkage in the cellulose molecule. It furnishes data regarding the minimal percentage of such a unit in any given sample, without, of course, giving any indication regarding the molecular magnitude of the cellulose. Cellobiose octaacetate has been prepared from cotton, wood (Wise and Russell, 1923), Spanish moss (Meer and Wise, 1935), and the rhizomes of brake fern (Harlow and Wise, 1938).

The purpose of the present study was to see whether the octaacetate could be obtained from a typical Bryophyte. The common hair cap moss *Polytrichum* sp. was selected, and to secure relative homogeneity, only the setae or capsule stalks were analyzed. The technique was the same as that previously reported (Harlow and Wise, 1938), hence only the results are summarized in the accompanying table. For the sake of comparison, the data from brake fern as well have

mination was made on a one gram sample of the alpha cellulose.

These data indicate that there is the same anhydro cellobiose linkage in the cellulose of the moss as that already found in the Pteridophyte and in the higher plants.

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WALLACE AND CLUM, "LEAF TEMPERATURES": A CRITICAL ANALYSIS WITH ADDITIONAL DATA¹

Otis F. Curtis

WALLACE AND CLUM (1938) have recently published a paper on leaf temperatures in which they present many interpretations and conclusions which appear to me as incorrect and unsound. Since the writers have published other papers that are thoroughly sound and since many readers may read the summary only or may fail to recognize the fallacies in the interpretations given—for several of them are easily overlooked—and since several of these interpretations and conclusions directly contradict those I have reported, I feel rather obliged to write the present paper in order to clarify certain points relating to factors influencing the temperatures of leaves, especially the influence of transpiration and radiation.

Wallace and his colleagues have devised ingenious equipment by which one can obtain continuous records of various conditions such as radiation intensities, temperature, etc., which can be recorded by electro-metric methods. By the use of this equipment, Wallace and Clum have attempted to obtain continuous records of leaf and air temperatures as influenced by various factors. From records obtained in these experiments, they concluded, among other things, that transpiration commonly cools leaves by amounts ranging from 6° to 9°C.; that "there seems to be no doubt . . . that transpiration is necessary at times to prevent leaves or leaf parts normal to the sun from being injured"; that a leaf of *Helioopsis* in full sunlight "normally wilts every day and that the greatest cooling of 7°C. below air coincides with the period of most severe wilting"; that a leaf even if covered by vaseline may transpire rapidly enough to be cooled below air temperature by as much as 5°C.; that "at night leaves are seldom, if ever, below air temperature due to loss (by radiation) to the sky . . ."; that "vaselining failed to prevent transpiration in leaves," while "waxing leaves with a mixture of beeswax and rosin prevented transpiration . . ."; and that a leaf covered with this wax becomes heated to killing temperature in the sun solely because the wax prevents cooling by transpiration. An examination of the evidence and reasoning that has led to these conclusions, however, indicates that each of these conclusions as well as other conclusions of Wallace and Clum are entirely unjustified.

One of the major sources of error in their work is that the thermopile which they assumed was at air temperature was probably 2° to 7°C. above air temperature at least a part of the time, and thus their comparisons between leaf and air temperatures at these times were incorrect. That the thermopile was at times not at air temperature is clearly indicated by several independent sets of their own data, as well as by evidence from other sources. For example, in

part of their figure 1, the curve for "air" temperature fluctuates as frequently and as abruptly as the corresponding curve for leaf temperature, and the air fluctuations show greater extremes. In referring to these curves on page 84, they say, "Note how each little detail or variation in temperature is present in both curves, although their amplitudes may be different." These very curves indicate that they were not measuring the true air temperature, for the air is almost completely transparent to the radiations that were influencing the leaf temperature, and the air temperature, therefore, would not vary directly with changes in radiation intensity except as it was being heated by the leaves or other absorbing objects. The thermopile which they "suspended in the air directly behind and in the shade of the leaf" was probably absorbing radiation directly or was so close to a leaf or other object that it was being heated by it. As stated above, they point out "how each little detail or variation in temperature is present in both curves . . .", air and leaf. They go on to say "This point is very important because the leaf is reacting not in accordance with crudely integrated temperatures, as measured by thermometers, but is instead reacting to every rapid fluctuation, some of which doubtless escape even the recorder used here. When wind is blowing through the leaves this correlation between leaf and air temperature all but completely disappears." A more likely explanation for this lack of correlation when the wind was blowing is that the thermopile under these conditions was more nearly recording the true air temperature, but they fail to recognize this point. They speak of air thermometers as crudely integrating the fluctuating air temperatures, which is true of course when the air temperature fluctuates rapidly, although I am not convinced that it is a crude integration. As already stated, because air is largely transparent to radiation, its temperature does not change rapidly with changes in radiation intensity, except as it is locally heated by absorbing objects such as the leaves. The air therefore could hardly be warmer than the leaf which is absorbing the radiation. It seems more probable that the air thermopile was close to or in contact with some object receiving direct radiation or was in a heat trap, as between two leaves, though not necessarily touching either. If radiation intensity was constant, which is highly improbable, and the fluctuations were due entirely to changes in air currents, it is conceivable that curves of somewhat the same form as these curves could be obtained, but even then the leaves in direct sunlight could hardly remain cooler than the air by the amount indicated, although they might occasionally be below air temperature for a few seconds.

¹ Received for publication July 18, 1938.

In the same figure, No. 1, which gives temperature curves for July 4, the leaves in direct sunlight seem to be continuously below air temperature from about 11 a.m. to sunset. At 2 p.m., when the leaves were most wilted, the curve indicates a leaf temperature 7°C . below air temperature. A great deal is made of this observation, and Wallace and Clum interpret it as due to the cooling effect of transpiration, but, as I shall show later, evaporation could not possibly have brought the leaf temperature to 7°C . below that of the air. It is more likely that, in the wilted condition, the leaf was no longer normal to the sun and was therefore close or slightly above the true air temperature but not below it. In order to check my interpretation that the wilted leaf was probably near air temperature, I wrote to the weather bureau station at Storrs, Connecticut, where this experiment was carried out, asking if the station had data on the temperatures for that particular day and hour. It is interesting to note that the station records show a maximum temperature for July 4 of 80°F ., which occurred at 2 p.m., the same time as reported by Wallace and Clum, but this is 7°C . below the air temperature reported by them and 0.6°F . below the temperature of the wilted leaf which they claimed was 7 degrees below air temperature. It seems obvious from this that the thermopile supposedly recording the air temperature was warmer than the air and that the wilted leaf which they thought was 7°C . below air temperature was probably slightly warmer than the air. The station records were taken at a distance of nearly two miles, and of course the actual air temperature at the station may have been cooler, but as indicated by evidence presented later in this paper, the transpiration from a wilted leaf in the sun could hardly be sufficiently rapid to cool the leaf below air temperature, so this wilted leaf was in all probability slightly above the true air temperature. Other alternative explanations that might possibly account for the seeming low temperature of the leaf in the curves of fig. 1A are that, as a result of melting of the ice in the ice bath, the cold junction of the air thermopile may no longer have been submerged, or that the figure is mislabeled and the curves for air and leaf temperature should be interchanged. The authors have informed me that the latter interpretation is highly improbable but they also disagree with my other suggested explanations.

On page 85 they report observations on the temperature of a vaselined leaf of *Heliopsis* and make the following statement, "About 6 p.m. the direct rays of the sun no longer struck the leaf, and the temperature quickly dropped to 5°C . below the air. The story then became clear; transpiration was continuing in spite of the vaseline." They fail to realize that a vaselined leaf, though it may perhaps evaporate slowly, couldn't possibly transpire fast enough to lower its temperature 5°C . below that of the air. Thinking that this low temperature of the vaselined leaf was due to transpiration, and failing to realize that it must have been due to an excessively high

reading for their "air" thermopile, they tried two other methods for stopping transpiration. One method involved the sealing of a piece of clear photographic film over the lower surface of the leaf, and the other was to cover the leaf with a wax consisting of equal parts of beeswax and rosin. "A summary of these two methods at the end of the day showed that the leaf with the first method had an average temperature of 14.8°C . above air, while the second method gave 13.2° above air. This seemed to indicate no appreciable difference in the temperatures given by the two methods, but since the cellulose film method might interfere with convection (?) currents, the wax method was chosen." The fact that the two methods had very similar effects on leaf temperatures, and that I had previously demonstrated that heating behind cellophane envelopes (Curtis, 1936a) was due to heat trapping and almost entirely independent of any effect on transpiration, should have warned them that the high temperatures they were observing when leaves were waxed were not chiefly due to the effect of the wax on transpiration.

When the vaselined leaf gave a temperature reading of 5°C . below the thermopile supposedly giving air temperature, they assumed it was due to transpiration from the vaselined leaf and therefore used the beeswax and rosin because it gave higher temperature readings and was supposed to stop transpiration. They make no comment on the fact that for the curve of Aug. 1 (fig. 5) the waxed *Verbascum* leaf shows readings below air temperature at five different times between 2 and 4 p.m., and these readings ranged between 2° and about 5.5°C . below air temperature. The control leaf which was free to transpire did not drop as much below air temperature, although it dropped below air temperature somewhat more often. They also fail to explain how a waxed white flower of *Althaea* (fig. 6), when held normal to the sun, could have had a temperature continuously below that of the air between about 2:40 and 5:15 p.m. Presumably, if they had observed this with a vaselined leaf, they would have explained it on the basis of high transpiration. An obvious explanation is that the air thermopile was not at air temperature. This seems to be the best explanation, especially in those cases where they found that control leaves when normal to the sun, or wilted, vaselined, or waxed leaves, remained continuously below air temperatures when in strong sunlight. The occasional momentary dips below air temperature may have been due to fluctuating air currents. Their own data, as well as my demonstration in an earlier paper (Curtis, 1936b), indicating that other investigators had misinterpreted leaf temperature data because air temperatures were not correctly measured, should have warned them to check their method of measuring air temperature. The simple expedient of shading the air thermopile by almost any opaque object held at varying distances would have demonstrated this fundamental error. Although it is highly probable that the air thermopile was not at air temperature, a possible alternative is

that the thermopile touching the leaves was not giving correct readings.

Further evidence that Wallace and Clum were not obtaining correct air temperatures is given by their claim that a vaselined leaf was cooler than the air by 5°C. They explained this as due to the failure of vaseline to stop transpiration, but they failed to realize that, for at least a part of the time, the relative humidities under which they were probably working were not sufficiently low even to bring about lowering the temperature of a wet bulb by the amount they claim. That is, a moist surface free to evaporate at a maximum rate would not be cooled to the extent they claim for a vaselined leaf. For example, hygrothermograph records from the Storrs station show a temperature range from 73° to 80°F. between 2 and 4 p.m. for Aug. 1, and a relative humidity of 88 per cent. At this humidity and temperature the maximum cooling of the wet bulb would be 1.5°C., yet their curve for the control leaf of *Verbascum* exposed at right angles to the sun shows readings as much as 4°C. below air temperature. Such a temperature

split corks and paraffin wax into small vials holding about 6 cc. of boiled water. Each entire vial with its leaf was weighed at the beginning and again at the close of the experiment so the water loss could be accurately measured. By this method the transpiration rates from several sets of *Iresine Herbstii* were measured. For each determination leaves matched in sets of three—untreated controls, waxed, and vaselined—were used. The data are presented in table 1. From these data it is evident that vaseline decreased transpiration on the average by about 92 per cent (88 to 95 per cent), and wax reduced it about 95 per cent (91 to 98 per cent). Although the transpiration from vaselined leaves was nearly twice that from waxed leaves, the actual rates in both cases were extremely low and would therefore have no measurable effect on leaf temperatures, especially under variable environmental conditions.

In later experiments sets of 12 to 16 or 20 leaves, usually 4 controls, 4 with wax, 4 with vaseline, and in some cases 4 coated with a wax emulsion (containing 70 per cent carnauba wax and 30 per cent

TABLE 1. Comparative effects of vaseline and wax on transpiration of single matched leaves of *Iresine*. Loss in wt. in gms. per leaf. Averages of 3 for each figure.

Control	Wax	Loss as % of control	Vaseline	Loss as % of control	Time and condition hrs.
1.42	0.027	1.9	0.07	4.9	6.5 Lab., artificial light
1.56	0.140	9.0	0.190	12.2	7 " " "
0.88	0.033	3.8	0.040	4.5	6 Greenhouse, sun light
0.84	0.033	3.9	0.060	7.1	6 " " , raining
0.78	0.047	6.0	0.087	11.3	4 " " , cloudy and sun
Av. 1.10	0.056	4.9	0.089	8.0	

lowering could not have been brought about by transpiration even in a leaf shaded from the direct sun. Because much of the leaf surface is not open to free evaporation, yet does allow for heat transfer from the air to the leaf, it is impossible for normal transpiration to cool the leaf below air temperature as much as the wet bulb is cooled. Although the relation between the amount of evaporation from a wet bulb thermometer and its temperature lowering is not linear, preliminary experiments indicate that to bring about a lowering of 5°C., a wet surface must evaporate at the rate of 1.5 to 5 cc. per square decimeter per hour, depending especially on the rate of air movement. Rarely does even a fully turgid leaf transpire at such rates; certainly a wilted leaf with intact epidermis or a vaselined leaf could not transpire that rapidly.

Since the temperature of a vaselined leaf appeared to drop 5°C. below that of the air, Wallace and Clum assumed that vaseline did not stop transpiration. We have made many measurements of water loss from individual leaves of several kinds to test the relative effectiveness of wax and vaseline on the transpiration rates. Individual leaves were cut, and the petioles or pieces of attached stem were sealed by the use of

paraffin, no. 284B, Franklin Res. Co.), were placed under various exposures, and the temperature of each leaf was measured by a thermocouple threaded through the leaf in such a way that the tip 2 to 4 mm. pressed against the lower surface. Ten such experiments were carried out—four with chokecherry (*Prunus virginiana* L.), three with bean (*Phaseolus vulgaris* L.), and three with lilac (*Syringa vulgaris* L.). Some were exposed to sun in the open, and others were exposed to artificial light in the laboratory. The leaves and lights were so adjusted that each one received the same light intensity as measured with a Weston photometer. To keep the angle of exposure nearly uniform for all leaves and to prevent changes in angle due to air currents, all vials were mounted on an adjustable rack, and each leaf was held in position by adjusting it between parallel threads fastened at each end of the frame. The method of mounting the leaves and attaching the vials to the rack is indicated in figure 1.² The findings were

² I am indebted to Miss R. L. Shurtleff for the experimental data presented in table 1 and to my son, W. Edgar Curtis, for devising the method of handling the vials and for the experimental data presented in tables 2, 3, and 5, as well as for many other determinations not here reported in detail.

TABLE 2. Comparative effects of wax, vaseline and wax emulsion on leaf temperatures and transpiration.

Leaf	Light	No. leaves in each treatment	Duration hrs.		Control	Waxed	Vaselined	Emulsion	Air
Bean	Mazda lamp	5	6	Av. temp. °C.	33.2	39.0	35.9	—	25.7
				Av. transp. gm.	0.660	0.088	0.105	—	
				Loss rel. to control ..	100	13.3	15.9	—	
Lilac	Sun	11	3	Av. temp. °C.	25.3	29.2	28.2	27.7	23.3
				Av. transp. gm.	1.909	0.020	0.045	0.201	
				Loss rel. to control ..	100	1.0	2.4	10.5	
Cherry	Mazda lamp	5	3.5	Av. temp. °C.	28.5	31.3	30.0	28.8	25.7
				Av. transp. gm/d ² /hr	0.428	0.018	0.025	0.109	
				Loss rel. to control ..	100	4.2	5.8	39.5	
Cherry	Sun	5	2	Av. temp. °C.	36.6	40.7	38.9	37.7	28.5
				Av. transp. gm/d ² /hr	3.09	0.119	0.062	0.678	
				Loss rel. to control ..	100	3.8	2.0	21.9	

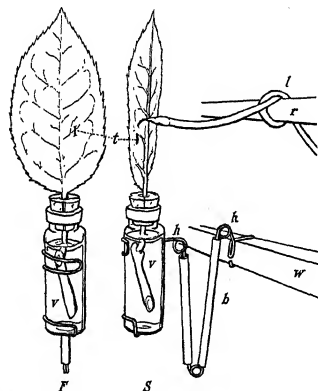


Fig. 1. Method of sealing leaf petioles into vials of water, *v*, and of mounting the individual vials in wire holders, *h*, which are stiffened with short pieces of bamboo, *b*, and attached to a wooden stick, *w*, which is firmly clamped on a heavy iron stand. The thermocouple, *t*, is threaded into the leaf blade so that the tip lies in contact with the lower surface of the leaf and from 5 to 10 mm. of the adjacent wire is also in contact with the leaf. The lead wires, *l*, which are encased in small rubber tubing are supported by a second wooden rod, *r*, clamped to the same iron stand. By bending the individual vial holders and rotating the vials, the orientation of each leaf can be easily adjusted to any desired angle, while the entire set of 12 to 20 vials can be moved as a unit by adjusting the iron stand. The vials can be lifted out of the wire holders at any time for weighing to determine the water loss. Face view, *F*, side views, *S*.

similar in all cases, and representative sets are presented in table 2.

It is to be noted that the control lilac leaves, though transpiring about ten times those coated with wax emulsion and about fifty times those coated with vaseline, average only about 2 to 2.5°C. cooler than the coated leaves. The control bean leaves, which transpired more than six times those that were vaselined, were likewise about 2.5 degrees cooler. The control cherry leaves transpired from 17 to 50 times the vaselined leaves, and yet were cooler by only 1.5 to 2.3°C. These same controls transpired 2.5 to 4.6 times those coated with wax emulsion, yet were cooler by only 0.3 to 1.1°C. The data obtained from these experiments with leaves covered with wax emulsion or vaseline as well as those obtained through the use of wilted or dried leaves, which are not here reported, are in agreement with findings from many sources that transpiration rarely lowers the temperature of leaves more than 2 to 5 degrees. The leaves covered with the beeswax and rosin mixture usually were from 1 to 3 degrees warmer than the vaselined leaves, but, as is shown in the following paragraphs, this heating was chiefly due to a change in absorption of radiant energy and not to differences in transpiration. The claim of Wallace and Clum that the wax mixture stops transpiration while the vaseline does not, for which they give no evidence other than the difference in temperatures of waxed and vaselined leaves, is not supported by the evidence here given. It is true that in all the experiments with *Iresine* as well as in the three with lilac, two out of three with cherry, and one out of three with bean, the leaves coated with vaseline lost slightly more water than those covered with wax, but the rates of transpiration were so very low from both that the slight difference in actual rates would have no measurable effect on temperature when exposed to natural conditions. It should be noted further that in these experiments

the wax coating was always thicker than the vaseline. From these data it is clear that the beeswax and rosin mixture is not much more effective than vaseline in reducing transpiration, while the necessity of applying it at high temperatures (about 62°C. or above) and its pronounced effects on changing the thermal properties of leaves make it highly unsuitable for studying the effects of radiation and transpiration on leaf temperatures. Although much more suitable than the wax, vaseline itself has several disadvantages; it probably alters somewhat the light absorbing, transmitting, and reflecting properties of a leaf as well as

light in some experiments and sunlight in others. The leaves were held in position by parallel threads as in the other experiments. Adjustments were made several times during the course of some of the experiments to make sure that differences were not due to inequalities of orientation or of position of thermocouple. The leaves were matched in sets of three and placed on the racks alternately in the order, control, waxed on upper surface, waxed on lower surface. Eleven experiments were carried out using the hypostomatous leaves of bean (*Phaseolus vulgaris*), cauliflower (*Brassica oleracea* var. *botrytis*),

TABLE 3. Effects on their transpiration and temperature of coating leaves with wax on the upper surfaces as compared with the lower surface. Leaves matched in sets of three. Each figure an average of determinations for four separate leaves. In most cases the temperatures are averages of ten or eleven readings for each of the four leaves.

Leaf and source of light		Control	Waxed on upper surface	Waxed on lower surface	Air
Cherry	Av. temp. °C.	21.7	23.3	23.3	18.5
Sun	Av. transp. gm.	0.471	0.935	0.091	
3.3 hrs.	Loss rel. to control	100	192.1	19.3	
5/17/38					
Cherry	Av. temp. °C.	28.4	30.4	32.0	22.6
Lamps 900 f.c.	Av. transp. gm.	0.965	2.169	0.164	
6.5 hrs.	Loss rel. to control	100	224.8	17.0	
5/13/38					
Bean	Av. temp. °C.	28.9	30.2	31.6	21.6
Lamps 800 f.c.	Av. transp. gm.	0.345	0.794	0.126	
3.5 hrs.	Av. transp. gm./d ² /hr. ...	0.421	0.969	0.154	
5/12/38	Loss rel. to control	100	230.2	36.5	
Bean	Av. temp. °C.	32.6	35.3	35.0	22.8
One 500 watt lamp	Av. transp. gm.	0.623	1.943	0.283	
Two 250 " "	Av. transp. gm./d ² /hr. ...	0.425	1.234	0.189	
at abt. 60 cm.	Loss rel. to control	100	290.4	44.5	
4 hrs.					
4/11/38					
Cauliflower	Av. temp. °C.	32.0	37.0	36.6	25.3
Two 500 watt lamps	Av. transp. gms.	3.183	1.753	0.803	
at abt. 60 cm.	Av. transp. gm./d ² /hr. ...	0.349	0.192	0.087	
4 hrs.	Loss rel. to control	100	55.0	24.9	
4/21/38					

its heat emission by radiation or conduction, while at high temperatures it is likely to melt and run and was also observed in some cases to injure the leaf.

Wallace and Clum concluded that the heating of waxed leaves was due solely to the fact that the wax prevented the cooling by transpiration. To test the effect on leaf temperatures we have made several sets of tests of leaves, some of which were waxed only on the upper surface, others only on the lower, while still others were left as controls. These leaves were placed with petioles in vials for measuring transpiration and with thermocouples attached to the lower surface for temperature measurements. The vials were arranged on a frame so that each leaf was exposed at right angles to a source of light, artificial

and cherry (*Prunus virginia*). One experiment with cherry leaves was not completed because the wax as applied was a little too hot, and the leaves were severely injured. The findings for each type of leaf were similar in all cases, and representative sets are presented in table 3.

These data clearly demonstrate that the waxed leaves are heated more than the check leaves, that this heating is largely independent of whether the wax is on the upper surface or the lower, and that this heating is also independent of whether the transpiration is high or low. It is especially interesting that those waxed on the upper surface showed transpiration rates in some cases two to three times those of the checks. They of course transpired more be-

cause the wax resulted in greater heating. This offers conclusive proof that the excess heating of the waxed leaves is not due to reduced transpiration, as claimed by Wallace and Clum, for the transpiration is greater and not less than that of the control leaves. That this is not merely a chance or temporary overheating of leaves waxed on the upper surface is demonstrated by the fact that this overheating was observed in averages of about ten readings for each of four leaves in each one of the ten sets of experiments.

It is true that Wallace and Clum report data (p. 89) purporting to show the comparative effects on leaf temperatures of waxing the upper surface as contrasted with the lower surface; but they report data from only one leaf waxed on the upper surface and one waxed on the under surface, and these were run on different days. The temperature of the one waxed on the upper surface was compared with that of the one waxed on both surfaces, while the one waxed on the lower surface was compared with an unwaxed control. Since the single leaf, waxed on both sides, on July 19 showed a maximum excess temperature of $8.5^{\circ}\text{C}.$ over that of the leaf heavily waxed on the upper surface only, while on July 20 the one waxed on the lower side showed a maximum of only $4.5^{\circ}\text{C}.$ warmer than a control with no wax, they concluded that waxing on the upper side had no effect on leaf temperature, while waxing on the lower side raised the temperature 4.5 degrees. (A leaf waxed on both surfaces showed a maximum excess temperature of $8.5^{\circ}\text{C}.$ over an unwaxed control on July 18.) This evidence is extremely weak, because it is based on only two leaves, one waxed on the upper surface and one on the lower surface, and each is compared with a different type of control on different days.

Furthermore, Wallace and Clum attempted to correct for what the temperature would have been if the leaves had had no stomates on the upper surface. That is, 80 per cent of the stomates were on the lower surface and 20 per cent on the upper, and they estimated that since blocking 80 per cent of the stomates had seemed to raise the leaf temperature $4.5^{\circ}\text{C}.$, if all the stomates had been on the lower surface and therefore all blocked with wax, the temperature would have risen $9.1^{\circ}\text{C}.$ Why they should reason that, if blocking 80 per cent would raise the temperature only 4.5 degrees, blocking the other 20 per cent should raise it more than twice as much (102 per cent) is hard to comprehend. Their method of calculation, though, is obvious. The control leaf that was free to transpire rose $14^{\circ}\text{C}.$ above air temperature. They disregarded this, however, and assumed that the leaf with 80 per cent of the stomates blocked with wax was $18.5^{\circ}\text{C}.$ above the air because four-fifths of the stomates were blocked and that the entire 18.5 degree excess was due to this blocking. From this they estimated the leaf temperature would have risen $23.1^{\circ}\text{C}.$ above the air if all the stomates were blocked ($18.5 = 80\%$ of 23.1). This method of calculation is entirely unjustified. Even assuming that transpiration is

directly proportional to the number of stomates, which is not a justifiable assumption, and neglecting, as they suggest, the greater reradiation at higher temperatures, there is no justification in calculating "as a linear function the heating which would have occurred had all the stomates been closed" Again on p. 91 they state " . . . from the temperature of a leaf waxed on the lower side only, one can correct for the number of stomates and calculate very closely what the temperature of the leaf should have been if both sides had been waxed." Such methods of calculation seem to demonstrate their complete failure to appreciate the factors influencing leaf temperatures; for when leaves are not at air temperature, heat transfer by conduction and convection may have an effect on their temperature far greater than can be brought about by transpiration and reradiation, and the temperature is not a linear function of evaporation. As shown in table 2 and the discussion of that table, control lilac leaves transpiring at very high rates, which were from 10 to 50 times those of vaselined leaves, were cooler than the latter by only about 2° to $2.5^{\circ}\text{C}.$ If a 5000 per cent increase in transpiration has a cooling effect of only 2.5 degrees, one could hardly expect that blocking 80 per cent of the stomates would raise the temperature $4.5^{\circ}\text{C}.$, and certainly blocking the remaining 20 per cent of the stomates could not result in raising the temperature another 4.6 degrees.

Even if the air temperature and the exact rate of transpiration from a leaf, or the rate of evaporation from a moist surface such as a wet bulb thermometer, were known, it would be impossible to estimate with any degree of accuracy the temperature of the leaf or that of the wet bulb, even if they were protected from direct sunlight. For example, in some preliminary experiments in which the rate of air flow was the only variable, while air temperature, barometric pressure, and relative humidity remained constant, I have found no direct relation between rates of evaporation and wet bulb lowerings. A representative set of data is shown in table 4. From these data it is clear that there is no constant relation between rate of evaporation and temperature of the wet bulb when the rate of air flow is the only variable. When one realizes the complexity of factors involved, an attempt to predict the temperature of a leaf in sunlight from an estimation of the number of stomates blocked or not blocked by the application of wax can at best be classed as naive.

The authors questioned by interpretation (Curtis, 1936b) of observations in which shielding leaves from the sky caused them to rise close to the air temperature from a temperature of 2° to $4^{\circ}\text{C}.$ below that of the air. I explained the rise in leaf temperature on the basis that the shield prevented radiation to a cold sky, and supported the interpretation by evidence from laboratory experiments with cold surfaces but not "in black boxes" as they state. (Field experiments on clear and cloudy nights that we have carried out here at Ithaca, N. Y., have confirmed my

earlier findings.) Wallace and Clum suggested that the shield merely reflected earth radiation. Unfortunately I did not mention the fact that in some of the experiments I measured the temperature of the surface of the soil and found this also to be cooler than the air, so their suggested explanation could not account for the observations. Furthermore, the shield used—heavy cardboard—would be a very poor reflector of infra-red.

sorption and radiation are at different wave lengths, there may be no relation between absorption and radiation. For example, white paint and white paper are poor absorbers of visible radiation but strong absorbers and radiators in the long-wave infra-red. An interesting summary table with source references giving properties of short-wave absorption and long-wave emission by many types of materials is presented in a bulletin by Brooks (1936). A failure on

TABLE 4. *Effects of altering wind velocities on rates of evaporation and temperature lowering of a wet bulb. Air temp. 23.9°C., rel. hum. 42 per cent, bar. p. 73 cm. Hg.*

Approximate wind velocities in ft/min.	15	190	340	465
Evaporation rates $\text{cc/d}^2/\text{hr.}$	2.5	6.3	9.2	11.6
Wet bulb lowering °C.	6.7	7.2	7.8	8.2
Ratio of lowering of wet bulb to evaporation rate	2.68	1.14	0.85	0.71

In attempting to determine radiation to or from the sky Wallace and Clum used a Shirley radiometer enclosed in a glass globe. The authors state, "Those junctions on the top of the grid are blackened to render them good absorbers, while those on the bottom are whitened to render them good reflectors." The black upper surface by absorbing from or radiating to the sky was assumed to indicate when these two processes were equal, while the whitened under-surface was assumed to reflect all radiation from the earth. From the use of this instrument they concluded that the zero exchange of energy between earth and sky occurred 20 minutes after sundown. They also attempted to make several other measurements of earth and sky radiation by the use of this instrument. They say, "This instrument is not suitable for quantitative determinations of gram calory values of earth and sky radiation, but it does offer a simple method of showing which of these is greater. It is used merely in this plus or minus sense in this work. The device is enclosed in a glass container which eliminates air currents." They failed, however, to realize that it is hopeless to use this radiometer in a plus or minus sense, because the instrument is very sensitive to the visible radiation which constitutes most of the "plus" from the sky, while it is highly insensitive to the infra-red at the wave lengths chiefly concerned in earth radiation and which constitutes the "minus." They assumed that the glass globe is as transparent to infra-red as to visible and that the whitened surface reflects infra-red as it does visible. Both of these assumptions are contrary to fact and entirely vitiate the interpretation of their attempted measurements of earth and sky radiation.

The failure of the authors to appreciate what they are measuring is further indicated by their statement on page 91 where they say, "... since if the wax rendered the leaf a poorer radiator, it would automatically reduce its absorption to the same extent, thus cancelling any effect." They completely overlook the fact that the absorption is chiefly in the visible and short infra-red, while radiation from the leaf is entirely in the longer infra-red. Where ab-

the part of Wallace and Clum to appreciate the basic principles involved is indicated again on page 96 where they say they used "white cardboard" as a reflector of infra-red, whereas actually in the long-wave, infra-red region, white paper is not unlike lamp black in its absorption and radiation properties. On the same page they make the following statement: "It is almost impossible in nature for any object to be so located that it does not intercept radiation from the earth, and since it does receive such radiation, it should not go below the temperature of the air, unless those bodies radiating to it are likewise cooler than the air." From this one wonders how they would explain the appearance of dew or frost on leaves and other natural objects that occurs so often on clear nights. Of course the leaves would be receiving some radiation from the earth, but they are in turn also radiating to the sky, and because of their low heat capacity and because of lack of heat conduction from the earth, they are more likely to be cooled to a greater extent than is, for example, the surface of the soil itself. When one realizes that the total earth radiation to the sky must on the average equal the total radiation absorbed, it becomes obvious that this radiation loss in the infra-red must be very great.

A point which Wallace and Clum may think I am overlooking is that I have been making temperature readings only at intervals, and since I do not have continuous records, may miss points of maximum difference which possibly exist for only very short periods. It is true that I may miss occasional points of maximum difference, but I maintain that in making comparisons between air and leaf temperatures, it is of greater importance to make certain that the true air temperatures are obtained than it is to catch occasional extremes. Furthermore, in studying the effect of transpiration on leaf temperature, it is necessary to avoid using methods for altering transpiration which have a greater influence on leaf temperature than has transpiration itself. Even assuming we had continuous records showing the exact temperatures of the air, of a freely transpiring leaf, and of a similar leaf with greatly reduced transpiration, we would not

be justified in concluding that the maximum differences obtained between the two leaf temperatures were due to differences in transpiration, for slight differences in angle of exposure or of air movements over the leaves may have much greater effects on leaf temperature than has transpiration. There may be no causal relation whatever between transpiration and occasional maximum differences in temperature.

On page 92 Wallace and Clum make the following statement: "Clum (1926b), table 2, found under the extreme conditions of an enclosed chamber that a plant in dry soil was consistently 7.9°C . warmer over a three-hour period than another plant in the same chamber which was in wet soil. Since the two plants were in the same chamber and therefore exposed to the same conditions, it is safe to conclude that this difference may be due to the wet and dry soil, or, in other words, available water." It is rather surprising that the authors feel it safe to conclude that this 7.9 degree difference was due to transpiration, when another pair of plants in the same experiment but in a dry chamber and with a transpiration difference between wet and dry plants more than four times that in the humid chamber (a difference of 1.82 gm/d²/hr. as compared with 0.43 gm/d²/hr.) had a smaller average temperature difference—that is, 5.6°C . A third pair of plants in the open had a transpiration difference very nearly the same (0.394 gm/d²/hr.) as that for the plants with a 7.9 degree difference but an average temperature difference of only 2.1 degrees. It is obvious from this that the differences in temperature in Clum's earlier experiments were not chiefly due to differences in transpiration. Although he measured transpiration from entire plants, he had thermocouples attached to only one leaf of each plant, and, although he tried to give the same exposure to both, obviously some factor other than transpiration was chiefly responsible for the differences in leaf temperature, as he himself pointed out in his earlier paper. One may wonder why Wallace and Clum made calculations of leaf temperatures from assumed differences in transpiration brought about by an assumed closure of 20 per cent of the stomates and yet failed to note that those leaves, from which transpiration data were at hand and which showed high transpiration rates in a dry chamber were less cooled than similar leaves in a humid chamber, even though the difference in rates of transpiration in the dry chamber was more than 400 per cent greater than in the humid chamber.

As stated above, there may be no causal relation between transpiration rates and occasional maximum differences in temperature. It would be just as "safe to conclude" that the minimum differences are a result of transpiration as it would be to select the maximum differences. Or perhaps in attempting to support the contention of one enthusiast for the omnigenous benefits of transpiration, one who has claimed that transpiration not only cools but also acts as a "temperature buffer," those instances in which a rapidly transpiring leaf is warmer than a

slowly transpiring leaf might be selected and this higher temperature ascribed to an effect of transpiration. Selecting maximum or minimum points and explaining them as due to one chosen factor, when several factors are involved, is of course entirely unjustified. With such marked individual differences and rapid fluctuations as occur when the environmental conditions are varying, it is clearly unsafe to depend on single maximum or minimum differences, or even on continuous uninterrupted curves, when they are records of only one or two leaves. Slight differences in angle of exposure, rate of air flow, thickness of leaf, and possibly position of thermocouple in a leaf, can introduce variables which may have effects on temperature greater than those due to transpiration. Even in a controlled non-fluctuating environment these variables will be important. It is practically impossible, especially under field conditions, to give two separate leaves exactly the same treatments, or treatments varying in one factor only. The errors resulting from these variables can best be eliminated by making large numbers of readings from different leaves. Occasional readjustments in leaf position and position of thermocouple may also help reduce the error resulting from these variables.

That transpiration is a relatively unimportant factor in dissipating the heat absorbed from incident radiation may be shown by calculations based on data concerned with intensity of radiation absorbed, rates of transpiration, and the heat of vaporization of water. At 20° , 30° , and 40°C . the heats of vaporization of water are respectively 584.9, 579.5, and 574.0 cal. per cc. Since the higher rates of transpiration are likely to take place at higher temperatures, and there is rarely danger of excess heating below 40°C ., I have taken the heat of vaporization of water at 40°C . for making the calculations. The heat of vaporization at 30°C . is only one per cent more and would therefore make no significant difference in the calculations anyway. On clear days a leaf with its surface normal to the sun will frequently be receiving incident energy at an intensity of 1.4 cal/cm²/min. or slightly more. It is of course at these higher intensities that there is greatest danger of the leaf becoming overheated. Although measurements indicate that leaves usually absorb more than 50 per cent of incident radiation, if we assume they absorb only 50 per cent of an incident intensity of 1.4 cal., they will be absorbing at the rate of 0.7 cal/cm²/min., or 4200 cal/d²/hr. Transpiration at the rate of 1cc/d²/hr. will absorb 574 cal., which is only 13.7 per cent of the absorbed energy. If the leaf absorbed 75 per cent of the incident energy, transpiration at the above rate would account for only 9.1 per cent. Thus, under the above assumptions, from 86 to 91 per cent of the absorbed energy must be dissipated by means other than transpiration. Transpiration rates may occasionally exceed the rate assumed, but when water is deficient, as it often is in the hot days of summer when there is the greatest danger of overheating, the rates are more often much less than this.

Wallace and Clum as well as others have claimed that leaves exposed to direct sunlight may be at temperatures of from 1° to 7°C . below air temperature and that transpiration is responsible for this low temperature. For transpiration to offset the heating effect of isolation and thus merely lower the leaf temperature to that of the air, it would be necessary to have extremely high rates of transpiration, rates probably much in excess of those commonly occurring in nature. For example, with incident radiation at $1.4 \text{ cal/cm}^2/\text{min}$.—that is, $8400 \text{ cal/d}^2/\text{hr}$.—if 75 per cent of this is absorbed by the leaf, it must transpire $10.8 \text{ cc/d}^2/\text{hr}$. to prevent a rise in temperature above that of the air, and if only 50 per cent is absorbed, it must transpire $7.3 \text{ cc/d}^2/\text{hr}$. To lower the leaf temperature even a degree or two below that of the

first mentioned experiments, which are the ones that show the greatest extremes, are presented in the upper part of table 5. Transpiration from the vaseline-leaved leaves varied between 0.025 to $0.062 \text{ gm/d}^2/\text{hr}$., while that from the control leaves varied from 0.428 to 3.09 . From these transpiration rates the rate of heat dissipation resulting from transpiration can be calculated. Such data are presented in the lower part of table 5. If the rate of absorption of incident energy were known, the fraction of incident energy dissipated by transpiration could be calculated. Unfortunately I have data on the approximate total incident energy for only one experiment, that with the cherry, which showed the highest rate of transpiration. These data were obtained from a recording pyrheliometer stationed within about 200 feet of the experimental

TABLE 5. Rates of transpiration and resulting heat dissipation from various leaves. Comparisons between control leaves and those waxed, vaseline, and coated with carnauba wax emulsion.

Plant	Light	No. leaves in each treatment	Rates of loss in $\text{cc/d}^2/\text{hr}$.			
			Control	Coated with beeswax and rosin	Coated with vaseline	Coated with wax emulsion
Iresine	Sun	3	0.450	0.018	0.032	—
Cherry	Mazda lamps	5	0.428	0.018	0.025	0.169
Cherry	Sun	5	1.353	0.030	0.035	0.224
Cherry	Sun	5	3.090	0.119	0.062	0.678

Heat dissipation by vaporization, $\text{cal/d}^2/\text{hr}$., and percentage of absorbed energy dissipated by transpiration, assuming incident energy at $1.4 \text{ cal/cm}^2/\text{min}$., and 50 per cent of this absorbed.

			Cal		Cal		Cal		Cal	
				%		%		%		%
Iresine	Sun	3	258	6.1	10	0.2	18	0.4	—	—
Cherry	Mazda lamps	5	243	5.8	10	0.2	14	0.3	97	2.3
Cherry	Sun	5	778	18.5	17	0.4	20	0.5	128	3.0
Cherry	Sun	5	1774	42.3	68	1.6	36	0.9	389	9.3

air, transpiration rates very much in excess of this must take place. So far as I have been able to find, rates of transpiration as high as $7 \text{ cc/d}^2/\text{hr}$. have been reported by only two investigators, and these rates were observed for short periods under very unusual conditions. These were maximum rates, and in one case the data indicate an error in the technique or calculation. Rates even as high as $1 \text{ cc/d}^2/\text{hr}$. are probably rarely, if ever, attained excepting when the leaf is in strong light and warmer than the air, and it is probably only when the leaf is warmer than the air that such high transpiration rates can take place.

In the present work the leaf areas were determined in four of the fourteen experiments in which both surfaces were waxed or vaseline, and in three of the twelve experiments comparing the effects of waxing the upper as contrasted with the lower surface. For these it was therefore possible to calculate the rates of loss per unit area and time. The data from the

leaves. The record indicates incident energy on a horizontal surface at an average of $1.33 \text{ cal/cm}^2/\text{min}$. for the two hours of the experiment. Although this is slightly lower than $1.4 \text{ cal/cm}^2/\text{min}$., this latter figure is assumed so as to make more ready comparison with the figures here given, and seems justified because maximum absorption must often equal or exceed the assumed 50 per cent absorption at times when transpiration is much lower than the exceptionally high figures reported in this paper for the control leaves.

Under the assumptions here made the heat dissipated by transpiration from the vaseline leaves would account for only 0.3 to 0.9 of one per cent of that absorbed. These extremely low figures for heat dissipation by transpiration from vaseline leaves should allay the fears of those who think the failure of a vaseline coating to raise the temperature of leaves

in strong light more than two or three degrees is due to its failure to stop transpiration.

Although the amounts of energy dissipated by transpiration can be fairly accurately calculated as above from measured rates of transpiration, the temperatures to which this transpiration will bring the evaporating surface cannot be thus calculated, even if the air temperature, the intensity of incident energy, and the percentage absorbed are known. Such calculation is impossible, because the loss by radiation and conduction (convection) would be variable and unknown.

From the evidence presented by Wallace and Clum it is clear that the high temperature attained by the leaves when covered with wax and exposed to direct sunlight resulted in death of the tissues. As already explained, however, the high temperature was certainly not entirely due to stoppage of transpiration. Furthermore, it is probable that the killing under wax was not due entirely to the high temperature. That is, the wax would interfere with gas exchange, and at the excessively high temperatures the unbalanced aerobic and anaerobic changes might be largely responsible for the death. The wax would interfere with diffusion of both O_2 and CO_2 , and would also tend to increase the moisture at the surface of the leaf. We have obtained some evidence indicating that high moisture, independently of its effect on cooling by transpiration, increases the susceptibility of the tissue to high temperatures. This may be comparable to the well known effect of moisture content of seeds on their resistance to heat, although the effect on leaves is much less marked than for seeds. Furthermore, under natural conditions the greatest danger of overheating usually comes at a time of day when water deficiency would not allow for the maximum cooling by transpiration; so, as I have previously stated, it seems that the time when cooling would be most helpful coincides with a time of little actual transpirational cooling and yet at a time of maximum danger of excess drying. Wallace and Clum are correct in pointing out that partial wilting may indirectly result in some cooling by changing the angle of the leaf to the incident radiation, but of course this change in angle not infrequently may result in greater absorption of radiant energy. Tropic responses to high intensities of radiation are more directly under the control of the plant as well as more effective in reducing leaf temperatures.

If Wallace and Clum were correct in their conclusion that transpiration commonly lowers the leaf temperatures by 6° to $9^\circ C$, they would be justified in their statement, "There seems to be no doubt . . . that transpiration is necessary at times to prevent leaves or leaf parts normal to the sun from being injured." Even a more probable cooling of only 2° to $5^\circ C$. may occasionally be a determining factor in life or death of a leaf when its temperature is close to the killing temperature, but we have obtained a fair amount of evidence that at these high

temperatures, the greatest danger is excess desiccation rather than thermal injury.

There are several points in the paper by Wallace and Clum, other than those I have mentioned, that are open to criticism, but they are less significant, so I shall not discuss them in detail. Some of these points concern the authors' method of measuring the effect of wax on transmission and reflection of visible and "total" radiation, their method of determining the possible effect of wax on leaf temperature by coating mercury thermometers, and their interpretation of these findings. Incidental and very minor corrections that should be classed as ordinary errata are as follows: Page 83, column 1, where they state that Watson found that "reradiation often accounts for more than 50 per cent of the heat loss from leaves," "emission" should be substituted for "reradiation," because Watson largely disregarded reradiation and investigated chiefly loss by conduction and convection and called it "emission." Page 91, in the last line of the caption for fig. 5, $52.5^\circ C$. should be substituted for " $19.5^\circ C$."

SUMMARY

From records obtained by the use of a recording instrument, Wallace and Clum have made several claims relative to the cooling effects of transpiration which can be refuted by a critical examination of their own data and also by evidence from other sources.

Their claims that transpiration commonly cools leaves by from 6° to $9^\circ C$, that a wilted leaf may be cooled by transpiration to $7^\circ C$. below air temperature when in sunlight at midday, and that a vase-lined leaf may transpire rapidly enough to cool it $5^\circ C$. below air temperature are shown to be unsound and based on what is probably faulty technique in measuring air temperatures.

Their claim that the heating and injury of leaves, when coated with a wax, consisting of equal parts of beeswax and rosin, and exposed to strong sunlight, results solely or chiefly from the elimination of the cooling effect of transpiration is shown to be unjustified, for evidence is presented showing that hypostomatous leaves, coated on the upper side by the mixture and exposed to strong light, become heated 3° to $7^\circ C$. above uncoated leaves given the same exposure, and yet at the same time may be transpiring from 50 to 290 per cent faster than the freely transpiring control leaves.

Their attempted calculation of what leaf temperatures would be if all stomates were blocked when based on measured temperatures of control leaves and leaves with stomates blocked on one surface only is shown to be fallacious. Not only does their method of calculation have no reasonable basis, but evidence is presented showing that with air velocity the only variable, the temperature of an evaporating body cannot be predicted even if the exact rate of evaporation, the air temperature, and relative humidity are known.

Their attempts to measure the relation between earth and sky radiation are completely vitiated because they used a radiometer which is sensitive to visible radiation and almost completely insensitive to the longer infra-red to which earth and leaf radiation is restricted. Their discussions of these and similar phenomena are unsound because they fail to appreciate that a material which may in one case be transparent to, or in another reflect, visible radiation, may be totally different in these properties when it comes to infra-red radiation.

Data are presented showing that vaseline, when applied to single leaves with petioles in vials of water, lowered the transpiration over three to six hour periods by about 85 to 95 per cent, while the wax mixture lowered it by about 90 to 98 per cent. The transpiration from leaves coated with a carnauba wax emulsion was reduced by from 60 to 90 per cent. The leaves coated with vaseline become heated only about 2°C. above the freely transpiring control leaves. The excess heating of leaves covered with beeswax and rosin mixture was due more to the effect of the wax on relative absorption and loss of radiant energy than to an effect on transpiration. The maximum rates of transpiration from vaselined leaves were so low that the heat dissipated by this transpiration could account for less than 0.5 per cent of an intensity of incident radiation to which leaves in full sunlight are frequently exposed. This demonstrates a fallacy in interpretation on the part of those who have claimed that the observed failure of vaselined leaves to heat more than 2 to 5 degrees in strong

light is due to the high transpiration from vaselined leaves.

Although the immediate effect of transpiration will invariably be to cool the transpiring tissue, its relative effectiveness in reducing the temperature or in preventing excessive heating has been greatly exaggerated. On the assumption that a leaf is absorbing 0.7 cal/cm²/min., which is only 50 per cent of that frequently incident on leaves exposed to the sun on clear days when there is greatest danger of overheating, transpiration must take place at the excessively high and unusual rate of 7.3 cc/d²/hr. if transpiration is to dissipate the absorbed heat and merely keep the leaf from rising above air temperature. To lower the temperature of a leaf below that of the air when the leaf is in direct sunlight would seem to require impossibly high transpiration rates.

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THE STYRAX OF NORTHERN CALIFORNIA AND THE RELATIONSHIPS OF THE STYRACEAE¹

Herbert F. Copeland

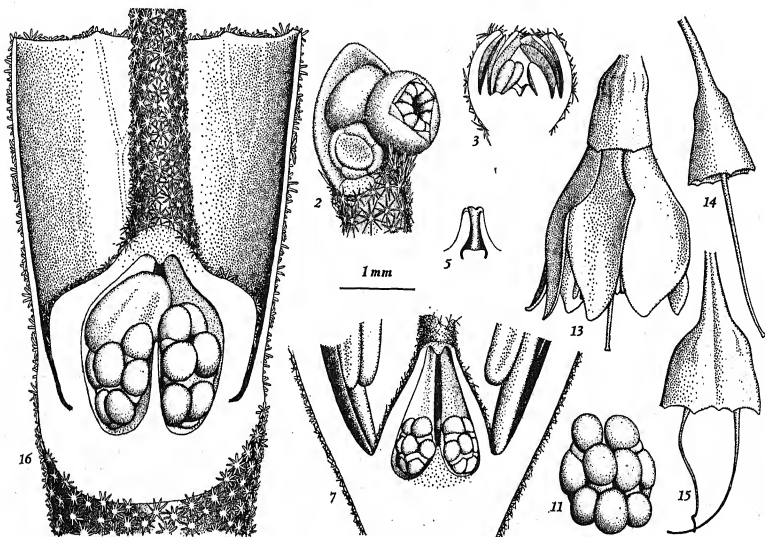
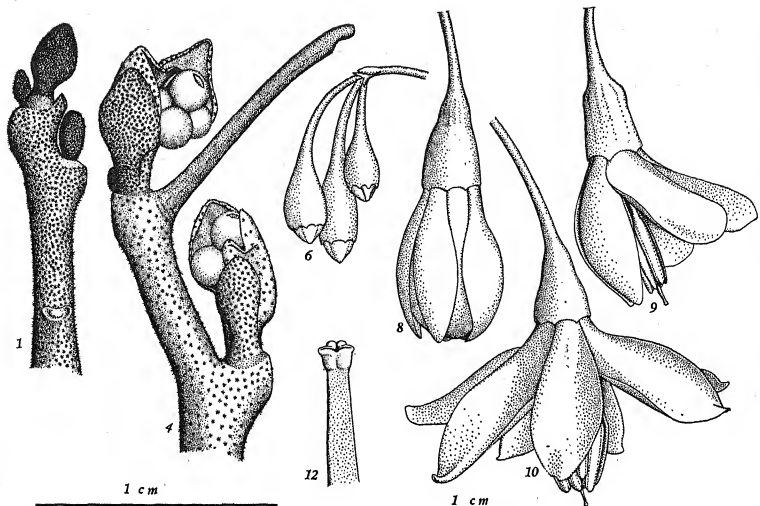
THE STATEMENT of Schnarf (1931) that the embryogeny of the Styracaceae is quite unknown, led me to make a general study of the form of *Styrax* which is native in Northern California. This form was described, from the collections of Fremont, by Torrey (1853) as *S. californica*. It was reduced by Perkins (1907) to synonymy with *S. officinalis* L. and was resurrected by Rehder (1915), and independently of him by Munz and Johnston (1924) as *S. officinalis* var. *californica*. These authors ascribe to *S. officinalis* an almost incredible distribution in the Mediterranean region and California and nowhere else, but the characters by which the Californian race has been distinguished from the Mediterranean seem neither constant nor significant. Having no acquaintance with the Mediterranean race, I follow the latest authorities and refer to the plant on which I have worked as *S. officinalis* var. *californica* Rehder.

The type locality is "upper Sacramento"; this means, presumably, the neighborhood of the junction of the Sacramento and Pitt Rivers, in the present

Shasta County, where the plant has repeatedly been collected. From Shasta County, its most northern locality, it extends in the Coast Range to Lake County and in the Sierra Nevada to the Mokelumne River, on the boundary between Amador and Calaveras Counties—mostly along watercourses in foothills not far from the Sacramento Valley. The area described is the northern half of Jepson's (1925) "Low Foothill" endemism area. Munz (1935) corrects Jepson's assignment of material from Southern California to this variety. The plant of Southern California is *S. californica* var. *fulvescens* Eastwood (1906), respectively *S. officinalis* var. *fulvescens* Munz and Johnston. It is distinguished by characters of no great weight, but fairly definite and constant.

I have studied the native plants chiefly at Folsom, in Sacramento County, at the very edge of the foothills, where they give way to the Sacramento Valley. From time to time—every week or two during the spring of 1936 and again during the spring of 1938—material was fixed in Bouin's fluid and in due course sectioned and studied microscopically. These studies

¹ Received for publication September 12, 1938.



have led to a hypothesis as to the relationships of the Styracaceae which will be presented below.

During the course of the work, I have had the benefit of correspondence with Dr. K. Schnarf and with Dr. I. M. Johnston and of conversation with Dr. I. W. Bailey and Dr. P. A. Munz. To each of these gentlemen I owe cordial acknowledgments, but the responsibility for the present paper is entirely my own.

GROSS FEATURES.—The plant is a deciduous shrub. Each winter bud (fig. 1) consists of an axis bearing, usually, three foliage leaves and a terminal inflorescence, all in an early stage of development. The outward, dorsal, sides of the leaves are beset with hard dark stellate hairs. Accessory buds, placed abaxially with regard to the axillary buds, are not uncommon (fig. 1, 4). The bud at the top of each figure is axillary and has an accessory bud to the left).

The winter buds unfold in February; the internodes elongate; the leaves expand and reach their full size late in March or in April. Meanwhile, the cymose inflorescence, usually of three flowers (fig. 6), is developing. In buds dissected in January, the flower primordia are mere knobs of tissue a fraction of a millimeter in diameter. Before the winter buds unfold, the synsepalous calyx and the separate corolla lobes and stamens develop more or less simultaneously (fig. 2). The calyx grows more rapidly than the other parts (fig. 3). By the time the winter bud opens and exposes the flower buds, it forms a globe about a millimeter across, almost completely enclosing the other parts (fig. 4). The pistil is first seen (fig. 3) as three little projections in the middle of the flower.

As the leaves reach their full size, the calyx reaches a length of about half a centimeter, and the corolla begins to project from it (fig. 6). The aestivation of the corolla lobes is imbricate; this is a character of the subgeneric group to which *S. officinalis* belongs (Perkins, 1907). By this time the brief tube, by which the corolla is rendered sympetalous and the stamens epipetalous, has begun to develop. The pistil has developed into a three-sided tube; the bottom of the lumen is expanded and is encroached upon by a mass of tissue which is to become the placentae (fig. 5).

The flowers open from the middle of April to the middle of May. A bud which is about to open is recognizable by a globular expansion of the corolla (fig. 8); at this stage, the style may or may not project to a distance of two or three millimeters. During a period of several hours, the lobes of the corolla swing back one by one (fig. 9). Full anthesis is reached at any hour of the day. The different flowers of a cyme open in basipetal succession during

several days. The individual plant shows an abundance of open flowers at all times during some two weeks. The flowers are pendant, white, some two or three centimeters across (fig. 10), and the flowering plant is very attractive.

The calyx is campanulate with the lobes reduced to obscure teeth.

Torrey stated the number of petals as six. Table 1 summarizes observations of numbers of petals and

TABLE 1. Number of flowers, among twenty selected at random, which show particular numbers of petals and stamens.

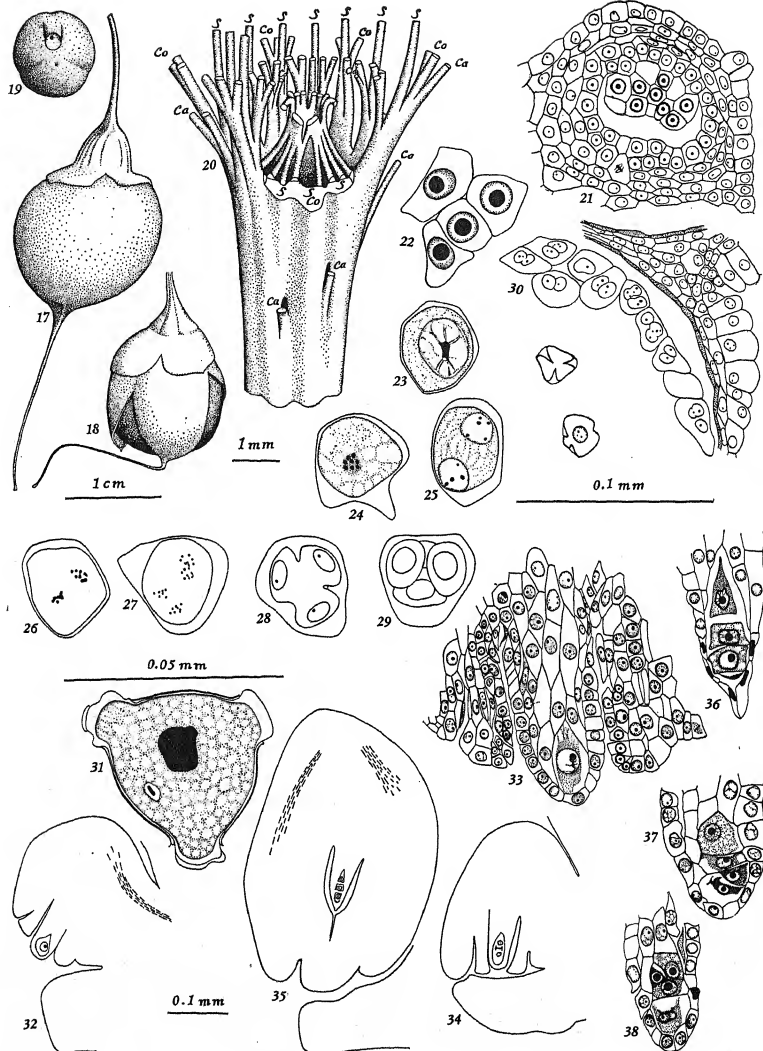
Numbers of stamens	Numbers of petals				
	6	7	8	9	10
11	1				
12	5	1			
13	2	2	1	1	
14		1			
15	1		1		1
16			1		
17		1			
18	1				

stamens as found in twenty flowers selected at random. It shows that these numbers are freely variable, that six is the most usual number of petals, and twelve of stamens, and that with number of petals greater than six, the number of stamens is usually less than twice as great.

The corolla is sympetalous, the tube about five millimeters long. To this distance the stamens are completely adherent to it. They are coalescent with each other, usually, to some distance beyond the level at which they become free of the corolla. In regard to this character, there is an amusing discrepancy between the statements of Torrey, "filamentis ad medium usque monadelphis," and of Rehder, "Staubfäden ungefähr ein Drittel verwachsen." The fact is, the distance to which the filaments are monadelphous is inconstant even in the single flower; a particular filament may be completely free of its neighbor on one side and united with the one on the other to a length of three millimeters, or half the distance to the anther. The lanceolate basifixed anthers, about 7 mm. long, bear the four linear pollen sacs on the margins and inner (ventral) surfaces.

The pistil is of three carpels. The ovary is partly inferior; approximately the lowest third of it is imbedded in the receptacle. As already mentioned, the pistil develops as a tube, open at the top, with placental tissue protruded into the bottom. Three septa

Fig. 1-16.—Fig. 1. Winter buds. $\times 5$.—Fig. 2. Flower buds within the swelling winter bud. $\times 15$.—Fig. 3. Section of a young flower bud. $\times 15$.—Fig. 4. Opening winter buds. $\times 5$.—Fig. 5. Section of pistil of bud. $\times 15$.—Fig. 6. Young flower buds. $\times 2$.—Fig. 7. Dissection of developing flower bud. $\times 15$.—Fig. 8. Flower bud ready to open. $\times 2$.—Fig. 9. Opening flower. $\times 2$.—Fig. 10. Fully open flower. $\times 2$.—Fig. 11. Placenta of open flower. $\times 15$.—Fig. 12. Stigma of open flower. $\times 5$.—Fig. 13. Withering of corolla. $\times 2$.—Fig. 14. Calyx from which the corolla has fallen. $\times 2$.—Fig. 15. Abnormal cohesion of pedicels and calyces. $\times 2$.—Fig. 16. Dissection of ovary some time after fertilization. $\times 15$.



grow from the walls radially inward. They reach the placental mass and become completely adherent to it, so that a cross section of the lower part of the ovary appears to show three chambers with an axile placenta in each. The septa do not meet, however, above the placentae; the three chambers are really one, continuous through the upper part of the ovary and continuous with the atmosphere through a three-winged channel the length of the three-sided style (fig. 7, 16). The stigma (fig. 12) is shallowly three-lobed; the lobes are alternate with the septa, each of them being the end of a single carpel.

The ovules are anatropous with the micropyles outward and downward—that is, they are apotropous. There are usually eight ovules on each placenta, two at the top, three in a middle row, and three at the bottom. The micropyle of each ovule opens upon an obturator, a massive outgrowth of the placenta; the obturators may or may not be laterally coalescent (fig. 7, 11, 16).

The outer surface of calyx and corolla and the ovary and lower part of the style are densely stellate-pubescent. The inner surfaces of calyx and corolla are sparsely stellate-pubescent, and there are a few stellate hairs on the pollen sacs.

The flowers have an exceedingly faint odor resembling that of the honeysuckle. Minute drops of liquid have been seen on the distal part of the ovary, but no taste of nectar could be detected. A variety of insects have been seen on the plants. Most of them seem to be fortuitous visitors. The only insects discovered to be active in pollination were honey bees. Several were captured while violently forcing their way in among the stamens.

Each flower remains open for about three days. Then the corolla lobes lose their turgidity (fig. 13), and corolla and stamens are shed (fig. 14). Figure 15 represents a rather common abnormality, the lateral coalescence of the pedicel and calyx of a terminal flower with those of the lateral flower next beneath it. The coalescence does not extend to the parts within the calyx.

Within a week after the fall of the corollas, many ovaries are visibly swollen. Many others (usually the ones farther from the terminal positions in the cymes) fail to develop: these fall from the plant, disjunction taking place at the base of the pedicel. Dissection of an ovary of which the swelling is just visible (fig. 16) shows always just one ovule developing. This is always one of the six ovules at the distal ends of the placentae. It develops in the part of the ovary which is free of the receptacle and where the inner margins of the septa are unsupported. It fills the available space, pushing the septa aside, and

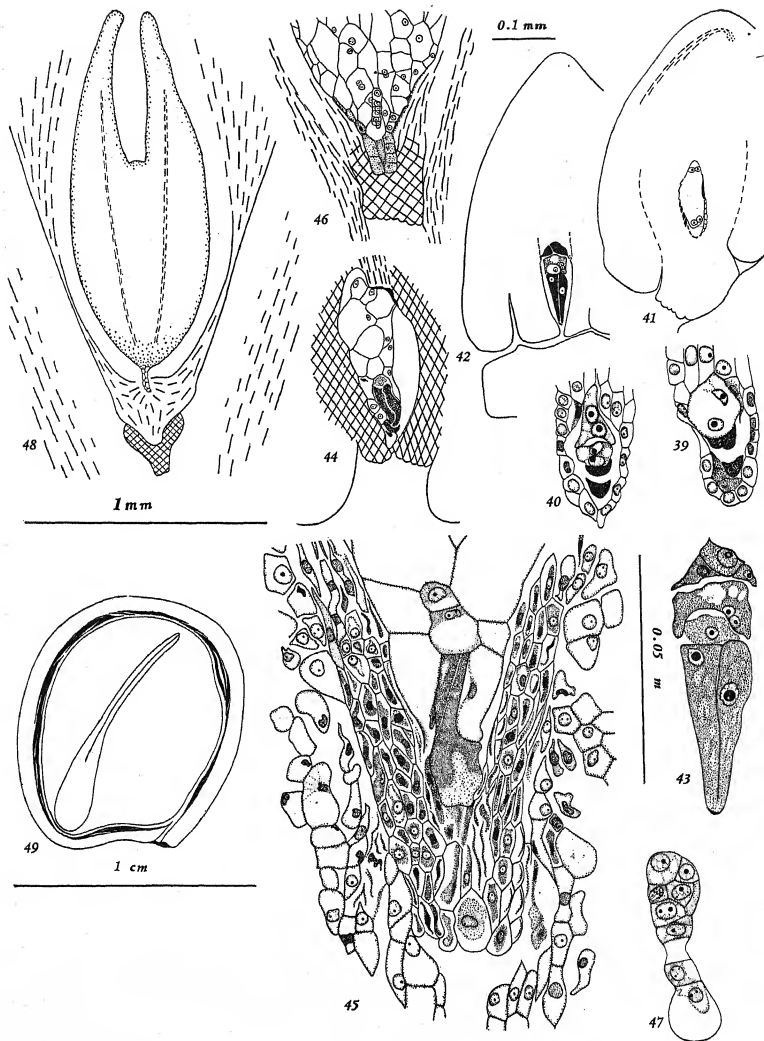
growing as the pericarp does. The ovary or young fruit presently so expands as to rupture the calyx and extend beyond it laterally as well as distally. The fruit (fig. 17) is ripe in August or September. The remains of the ovules which have failed to develop may still be detected within the proximal end of it. It opens by three sutures in planes alternate with the septa (fig. 18). It is, then, a loculicidal capsule. The seed (fig. 19) is chestnut colored, smooth, shining, subglobular, longitudinally grooved apparently by pressure against the septa. It has a conspicuous hilum, showing the stub of a vascular bundle leading into the prominent raphe, and an inconspicuous micropyle.

HISTOLOGICAL NOTES.—The following histological observations do not cover the whole plant. For the most part, they touch on characters which have been or may be found significant in comparative studies.

The arrangement of vascular tissue in the receptacle is illustrated in figure 20 (this is not drawn exactly from a single example, but is made up from a study of several). From the siphonostele in the pedicel, at the level of transition to the receptacle, there depart a number, typically six, of sepal bundles, leaving small gaps, which are quickly closed. There are no lateral bundles to the sepals. Above (distally with regard to) their departure, the stele becomes radially lobed, the lobes alternating with the sepal bundles. At this level, a whorl of a considerable, indefinite number of bundles departs into the pith; these draw together in the lower part of the ovary into a more or less continuous, triangular-prismatic structure. The angles are directed toward the placentae. The continuous body breaks up distally into separate bundles which enter the ovules. As to the main body of the stele, each lobe divides periclinally into an outer petal bundle and an inner stamen bundle. The tissue between each two lobes becomes a stamen bundle. From the inner and lateral sides of the stamen bundles arise an indefinite number of bundles which turn inward and ascend the wall of the ovary. From among these ovary wall bundles arise three smaller bundles located respectively in the middle radial planes of the locules. These are the carpel-dorsals. They are located slightly more deeply within the walls of the ovary than the other bundles, and while all the rest fade out at the summit of the ovary, these enter the angles of the style, ascend it to the summit, and fade out in the lobes of the stigma. In all details of the vascular system just described, there is much variation and irregularity.

The receptive surface of the stigma is a continuous layer of columnar cells with the free ends domed

Fig. 17-38.—Fig. 17. Fruit. $\times 2$.—Fig. 18. Dehiscent fruit. $\times 2$.—Fig. 19. Seed. $\times 2$.—Fig. 20. Reconstruction of vascular system in the receptacle. About $\times 20$. *Ca*, sepal bundles; *Co*, petal bundles; *S*, stamen bundles; *Cd*, carpel-dorsals.—Fig. 21. Cross section of pollen sac in young bud. $\times 400$.—Fig. 22. Pollen mother cells. $\times 900$.—Fig. 23-29. Stages of development of pollen mother cells into pollen grains. $\times 900$.—Fig. 30. Cross section of pollen sac shortly before anthesis. $\times 400$.—Fig. 31. Pollen grain. $\times 900$.—Fig. 32. Ovule of young bud. $\times 125$.—Fig. 33. Nucellus from fig. 32. $\times 400$.—Fig. 34. Ovule showing first division of megaspore mother nucleus. $\times 125$.—Fig. 35. Ovule showing megaspore tetrad. $\times 125$.—Fig. 36. Megaspore tetrad from fig. 35. $\times 400$.—Fig. 37-38. Megaspore tetrads. $\times 400$.



outward; these cells are not distinguished by staining reactions.

Each epidermal hair originates as a single epidermal cell which grows so as to project beyond the general surface level. It divides by walls in planes which include or are parallel to its own long axis—that is, at right angles to the plane of the epidermis (an early stage in the development of a hair is shown in fig. 21). Each of the approximately eight resulting cells grows out in a plane more or less parallel to the plane of the epidermis and becomes one ray of the hair. The cell walls become thick and heavily stainable with saffranin.

Sclerenchyma, of large cells with thick, pitted, lignified walls, begins to appear outside of the vascular cylinder in the pedicel and receptacle some weeks before the flowers open. Subsequently it appears in the calyx and the wall of the ovary and eventually in the seed coat. In pedicel and receptacle, the cells are generally elongate—that is, of the character of fibers. In ovary and seed coat they are more or less isodiametric—that is, of the character of stone cells. Between these there is a complete series of intergrades. The sclerenchyma, in combination with the epidermal hairs, renders buds, flowers, and fruits, as whole objects, very refractory to sectioning on the microtome.

The wood exhibits annual rings. Of these, each of the first few may add nearly a millimeter to the diameter of the stem; the later ones are much narrower, and a stem 15 mm. in diameter may be twenty years old. In the primary wood, the vessels are narrow and scattered, with highly oblique scalariform perforations. Early in each annual ring, there is a circle of large vessels with porous perforations which are nearly transverse. In the remainder of each of the earlier annual rings, there are scattered smaller vessels resembling those in the primary wood. The later, much narrower rings afford no room for these. Wood rays are numerous, about three cells wide. Wood parenchyma occurs as tangential bands, one cell deep, extending from one ray to another. Between each two bands of parenchyma there are some two or three layers of wood fibers. Rough measurements of the wood elements are given in table 2.

Occasion was taken to observe a character already recorded by Sinnott (1914), the structure of the node. Each leaf is supplied by a single vascular bundle, leaving a single gap in the stele.

DEVELOPMENT OF STAMENS AND POLLEN.—The stamens and pollen grains show no striking peculiarities. Some five or six weeks before the flowers open, the stamens being some two millimeters long, the cross section of a pollen sac (fig. 21) shows the usual epidermis and two more layers of wall cells, tapetum, and mass of pollen mother cells. The pollen mother

TABLE 2. Dimensions of wood elements in microns.

	Length	Width
Wood parenchyma	c. 50	16
Wood fibers	650—800	16
(Lumen of wood fibers)	—	(8)
Vessels with oblique scalariform perforations	300—400	30—50
Vessels with porous perforations	300—400	80—150

cells are large, with large nuclei and nucleoli. They remain in this condition for two or three weeks (fig. 22). Then, in the course of a few days, they develop thick walls and go through the usual stages of pollen formation (fig. 23–29). The haploid chromosome number is eight; the chromosomes are all alike in appearance, little globes which decrease in size as they divide, until at the end of the homeotypic division (fig. 27) they are of the order of one-quarter micron in diameter. The pollen grains are formed by simultaneous furrowing (fig. 28). The tapetal cells, meanwhile, have enlarged and become binucleate (fig. 30). As the pollen grains enlarge and mature, the tapetal nuclei become enlarged, lobed, and only vaguely stainable. Finally the tapetum and the innermost layer of wall cells collapse and become unrecognizable.

Along the line of contact of the two pollen sacs of each anther lobe, the epidermis becomes differentiated, by enlargement of cells, as a stomium. In the cell-layer next within the epidermis, the cell walls become ribbed. The ribbing appears late, after the pollen grains are formed, and it is not strongly developed. It extends along the exposed surface of each pollen sac only about two thirds of the distance to the stomium, and the ribs are mere rods or pillars, not complete hoops.

The maturation of the pollen grains takes place during some two or three weeks after their formation. The mature grain is globular, three-grooved, with yellow droplets on the surface. The contents stain heavily and are definitely alveolar in structure. It was with some difficulty that nuclei were recognized (fig. 31). The large, more or less globular, tube nucleus stains intensely under various treatments, but not distinguishably with iron acetocarmine. The small ellipsoid generative nucleus is seen, not without searching, after staining with either iron acetocarmine or iron haematoxylin. These observations agree with those of Schnarf (1937) on *Halesia*, in which no generative cell could be distinguished.

Pollen grains were suspended in hanging drops of a series of solutions of sucrose. In water, 1 per cent sucrose, and 5 per cent sucrose, the contents presently burst through one pore of each grain. In twice molar

Fig. 39–49.—Fig. 39–40. Binucleate embryo sacs. $\times 400$.—Fig. 41. Ovule showing 4-nucleate embryo sac. $\times 125$.—Fig. 42. Ovule showing embryo sac ready for fertilization. $\times 125$.—Fig. 43. Embryo sac from fig. 42. $\times 400$.—Fig. 44. Section of endosperm and zygote, approximately same stage as fig. 16.—Fig. 45. Two-celled embryo and surrounding structures. $\times 400$.—Fig. 46. Suspensor and surrounding structures. $\times 125$.—Fig. 47. Developing embryo. $\times 400$.—Fig. 48. Part of section of developing seed. $\times 50$.—Fig. 49. Longitudinal section of mature seed. $\times 5$.

(68 per cent) sucrose, they shrank in the equatorial plane (the plane at right angles to the long axes of the grooves) and became ellipsoid. In solutions from 10 per cent to 34 per cent (the latter being molar) sucrose, they appeared normal, being about fifty microns in diameter. In each groove, the middle part of the surface is covered by an elliptic plaque, while at the ends the intine is exposed. Germination was evident in less than an hour in every solution in which the grains were of this appearance. Contamination by yeasts prevented the carrying through of this experiment to observations of the rate of growth and internal structure of the pollen tubes.

DEVELOPMENT OF OVULES AND SEEDS.—The ovules are first recognizable, in March, as obscure knobs on the placenta. In April, when pollen grains have been formed but are yet to mature, the ovules, about one-fifth of a millimeter long, show their apotropous character (fig. 32). The two integuments are separate only to a short distance back of the micropyle. The nucellus is a column several cells thick. One cell, just within the epidermis at the free end, is enlarged and includes a nucleus apparently in synapsis (fig. 33). This is the archesporial, and at the same time the megaspore mother, cell. It develops, during the next two or three weeks, in normal fashion into an embryo sac, the ovule meanwhile growing to a length of about half a millimeter (fig. 42). The megaspore tetrad is in some examples (fig. 35, 36) lineal; in others it is T-shaped (fig. 37) or inverted T-shaped (fig. 38). The megaspore which develops is ordinarily the chalazal one (fig. 39). In one ovule, it was found that the one next to this had also begun to develop (fig. 40). The developing embryo sac absorbs and projects beyond the single nucellar layer of cells at the micropylar end. The chalazal part of the nucellus remains as a sort of pedestal under the embryo sac (fig. 42). The inner integument, as far as it is distinct, and the inner layers of cells where there is no definite boundary between the two integuments are by this time visibly differentiated from the outer layers. The cells are more closely packed and more stainable. (In figure 45, I have attempted to show this differentiation as it appears at a later stage.) The synergids are notably elongate and heavily staining; the egg, hidden among the other cells, is hard to distinguish; the endosperm mother cell is binucleate, approximately isodiametric, full of stain-resistant granules; the antipodals are rather small and stain heavily (fig. 43). Among the more than a score of ovules in each ovary, only one of which is to develop into a seed, there is no evident differentiation.

Regrettably, fertilization and the earliest stages following were not observed. A week or two after fertilization (fig. 44), the developing ovule having grown to a length of about a millimeter, the endosperm is cellular, showing in longitudinal section about a dozen cells, as if it consisted altogether of about two dozen. I suppose that it is cellular from the beginning. It is evident that it is acting destruc-

tively upon the antipodals, the nucellar pedestal, and the inner cell layers of the integuments.

The zygote remains undivided for about a month after fertilization. By this time, the outer integument has grown very considerably. It has extended forward and closed over the inner integument, but there remains a long and slender micropylar passage through it. Stone cells have developed in the layers within the epidermis, and inward from these there is a layer of vascular bundles, branches of the bundle which enters the ovule through the funiculus. The endosperm has developed to some extent. Of the inner integument there remains only a detached mass of tissue as a cap over the micropylar end of the endosperm. This cap persists for some time (fig. 46, 48).

Subsequently, the zygote becomes a uniseriate suspensor six or eight cells long (fig. 46), the distal cells divide longitudinally, making the suspensor pluriseriate (fig. 47), and the terminal cells develop into the dicotyledonous embryo (fig. 48).

In the seed (fig. 49), the integuments are absorbed as far as the vascular bundles, which form a fibrous layer. Outside of this layer, the seed coat is essentially a mass of stone cells, forming a hard and brittle shell. The endosperm is of large and thin-walled cells containing no starch but much oil, and is almost tasteless, slightly astringent. The large elliptic cotyledons lie in a plane at right angles to the plane of symmetry of the seed. There is a massive hypocotyl from which the radicle cannot be distinguished. A plumule can scarcely be detected.

THE RELATIONSHIPS OF THE STYRACACEAE.—The family most closely related to *Styracaceae* is *Symplocaceae*, which is, in fact, a segregate. In most systems (Wettstein, 1911; Engler and Gilg, 1924), *Styracaceae* and *Symplocaceae* are placed next to *Sapotaceae* and *Ebenaceae*, these four, with certain obscure minor families, constituting the order *Ebenales* or *Diospyrales*. Baillon (1867-1894), however, and Hutchinson (1926), maintain that *Styracaceae* and *Ebenaceae* are not closely allied.

In the structure of the wood, *Ebenaceae* are decidedly different from *Styracaceae*: their wood parenchyma tends to be gathered about the vessels, and the perforations in these are exclusively porous (Solender, 1908). Such differences do not preclude the possibility of relationship; they are marks of relative evolutionary advancement (Frost, 1930), *Ebenaceae* being in this respect more highly evolved than *Styracaceae*. In *Sapotaceae*, as represented by *Bassia latifolia* (Jones, 1924), the wood is very much as in *Styracaceae*.

Ebenaceae have pistils of four carpels, with just two apotropous ovules to the carpel and no obturators; the two integuments of each ovule are distinct down to the chalaza. These characters may be evaluated as of about the usual weight of distinctions among the families of an order. Embryologically (as to *Ebenaceae*, see Hague, 1911; Yasui, 1915; Schnarf, 1931), the two families are decidedly similar. In two particular details, the presence of a pedestal-like

remnant of nucellus under the embryo sac and the delayed development of the zygote, there is a striking identity. The Symplocaceae also, so far as they are known (Schnarf, 1931), are in good agreement. They have only one integument. This may have arisen by the fusion of two integuments, a process which seems to be under way in Styracaceae. It appears that the authors who have established and maintained the order Ebenales as generally understood are justified; the group is a natural one.

When I began the study of *Styrax*, I expected not to find many points of resemblance to Ericales. This expectation was mistaken. The pubescence of *Styrax* is not unlike that of *Rhododendron*. Styracaceae and Ericaceae (or at least Monotropoideae) have the outer whorl of stamen bundles associated with petal bundles. An open style passage is a character of Ericales. The poor development of ribs in the wall of the pollen sac of *Styrax* recalls their absence in Ericales, and, of course, the tenuous nucellus and the archesporial cell which is itself the megaspore mother cell are shared by these two groups as by most Sympetalae. Hayata (in Matsumura and Hayata, 1906) actually recognized the Formosan genus *Alniphyllum* as a direct intermediate between Ericaceae and Styracaceae; but he deceived himself, the plant being perfectly at home in Styracaceae and by no means placeable in Ericaceae. What these observations suggest is not a direct but a collateral relationship between the two groups.

The ancestry of the order Ericales is fairly well established by consensus of opinion. It is represented among living plants by *Saurauia*, a genus of Actinidiaceae (Lindley, 1846; Hutchinson, 1926; Copeland, 1932; Schnarf, 1933). Dilleniaceae, Actinidiaceae, Theaceae, Guttiferae, and Dipteroocarpaceae, with certain minor families, make up the order Theales of Hutchinson, the order Guttiferales of Wettstein, the suborder Theineae of the Parietales of Engler. With all the foregoing data in mind, I anticipated that one or another of the families of Theineae might turn out to be more closely related to the Styracaceae than the Ericaceae are. This anticipation seemed justified as soon as Theaceae were considered. The following characters of Theaceae, in part as seen in locally cultivated plants of *Camellia japonica*, in part as stated in the literature, were noted:

Gross characters (von Syszolowicz, 1895): flowers slightly sympetalous, stamens slightly monadelphous, carpels often three, fruit a loculicidal capsule.

Wood (Solleder, 1908): vessels all with oblique scalariform perforations; rays numerous, all or mostly narrow; parenchyma mostly in tangential bands from one ray to another. Kanehira (1921; referring to the woods only) remarks that "Ternstroemiaceae, Ericaceae, and Styracaceae closely resemble each other." Nodes (Sinnott, 1914): unilacunar (as in other Theineae, and also all Ericales except Epacridaceae).

Embryogeny (Cavara, 1899; Cohen-Stuart, 1916; Schnarf, 1931): Pollen grains three-grooved; ovules bitegmous, tenuinucellate, the female archesporial cell

itself the megaspore mother cell. "Der Nuzellus . . . wird in seinem oberen Teile vom heranwachsenden Embryosacke aufgezehrt." Development of the zygote is long delayed. Theaceae differ from Styracaceae in having exalbuminous seeds, and possibly also in the early development of the endosperm. Schnarf states that this is in *Thea sinensis* of the nuclear type; but the accounts of Cavara and Cohen-Stuart, his main authorities, seem to me not to establish this point. As noted, I am not perfectly certain of the situation in Styracaceae. In Ebenaceae, the endosperm is definitely of the cellular type.

The facts here assembled seem to establish a strong probability that the Theaceae are the living plants which most nearly represent the ancestry of the Ebenales.

SUMMARY

Styrax officinalis var. *californica* Rehder (*Styrax californica* Torrey) is a shrub of the foothills about the Sacramento Valley.

The wood shows vessels with porous perforations in the early part of each annual ring; vessels with oblique scalariform perforations elsewhere; narrow wood rays, and tangential bands, one cell deep, of wood parenchyma.

The winter buds, each covered by only about three young foliage leaves, open in February; leaves are mature in March or April; the abundant handsome white pendant flowers, arranged in cymes, are open in April or May.

The aestivation of the corolla is imbricate. The petals are more or less six, the stamens more or less twice as many. These parts are united into a brief tube; the stamens are monadelphous to a varying distance beyond the level at which they become free of the corolla.

The development of stamens and pollen is quite normal. The ribbing of the endothecium of the pollen sac is poorly developed. Tapetal cells become binucleate. The haploid chromosome number is eight. Pollen grains, originating by simultaneous furrowing, are three-grooved, binucleate.

The pistil is of three carpels, with partitions which reach the placentae below but do not meet above; the style is transversely by an open channel. Each placenta bears about eight apotropous bitegmous tenuinucellate ovules equipped with obturators. The female archesporial cell is itself the megaspore mother cell. Development of the embryo sac is of normal type. In any one ovary, only one ovule develops beyond this stage.

The endosperm is probably cellular from the beginning. The development of the zygote is delayed for about a month. The endosperm absorbs the inner integument (a remnant persists for some time at the micropylar end) and most of the outer integument. The zygote forms a suspensor of which the distal part becomes pluriseriate; from the end of this, the large dicotyledonous embryo develops.

The fruit, a loculicidal capsule, is ripe in August or September.

These characters substantiate the position of the Styracaceae in the order Ebenales; and indicate that

the living plants which most nearly represent the ancestry of the Ebenales are the Theaceae.

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THE FLORAL ANATOMY OF THE AURANTIOIDEAE¹

Albert H. Tillson and Ronald Bamford

A KNOWLEDGE of the relationships of the cultivated and wild species of the Aurantioideae, as well as of other groups which contain commercially important plants, is desirable from the standpoint of plant improvement by breeding or grafting. Many lines of attack on the problems of relationships are being followed. The present study was undertaken to ascertain what light could be thrown on the unsettled question of the systematics of the Aurantioideae by anatomical investigations.

There has been very little study of the vascular anatomy of the citrus fruits. Among the earlier investigators, Penzig (1887) mentions the bundle traces of a few species in a brief and incomplete way, but makes no attempt to use them in a systematic plan.

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Saunders (1934) made a study of the vascular systems of 33 species of rutaceous plants, five of which are in the Aurantioideae, but facts which may be of systematic value are overlooked in an attempt to prove her theory of carpel polymorphism. Furthermore, the 25 genera studied could hardly be considered representative of a family composed of more than 140 genera.

Taxonomic studies on the Aurantioideae made by early workers were based largely upon the number of stamens, locules, and ovules of the comparatively few species known at that time. These characters, as pointed out by Tanaka (1936), may vary even within a single genus. In later years Engler (1931), Tanaka (1936), and Swingle (in press) have made use also of such characters as type of inflorescence, type of leaf, number of leaflets, presence of winged rachis and petiole, number of thorns on the stems, number and form of floral organs, formation of pulp

vesicles and oil glands, and hardening of the rind of fruits.

Engler (1931), revising his earlier classification (1896), accepts many of the new genera proposed by Swingle (1914, 1938) or lists them as subgenera. The former considers the Aurantioideae to consist of only one tribe, the Aurantieae, which he divides into the subtribes Hesperethusinae and Citrineae.

Tanaka (1930) groups the genera of the Aurantioideae into eight tribes: Micromelaeae, Clauseneae, Aegleae, Lavangeae, Meropeae, Atalantieae, Microcitreae, and Aurantieae. He fails to accept four of Engler's genera, regarding *Thoreldora* and *Tetracrona* as species of *Glycosmis*, *Lamiofrutex* as a species of *Atalantia*, and *Afraegle* as a section of *Balsamocitrus*. *Chalcas* is used in preference to the generic name *Muraya*. Tanaka also recognizes the division of the genus *Atalantia* by Swingle (1914) into the five separate genera: *Atalantia*, *Severinia*, *Pamburus*, *Paramignya*, and *Merope*. He also adds the genera *Ozantha* and *Pleurocitrus*, both of which were previously included as species of *Citrus*.

Swingle's classification (in press) of the tribes and subtribes of the Aurantioideae does not entirely agree with either of the above. He divides the subfamily into two tribes: Clauseneae, with the three subtribes Micromelinae, Clauseninae, and Merrillinae; and Citreae, with the three subtribes Triphasinae, Citrinae, and Balsamocitrinae. He further suggests the subdivision of the subtribes of the Citreae into smaller groups, and proposes three new genera: *Burkillanthus* (*Citrus malaccensis*), *Clymenia* (*Citrus polyandra*), and *Limnocitrus* (*Pleiospermium littoralis*). Swingle's classification is used below, since it is more nearly in agreement with the findings of this study than are the other systems mentioned.

MATERIALS AND METHODS

The materials upon which this study is based include floral buds, ovaries, and young fruits of the following 94 species comprising 29 of the 33 genera which make up the orange subfamily:

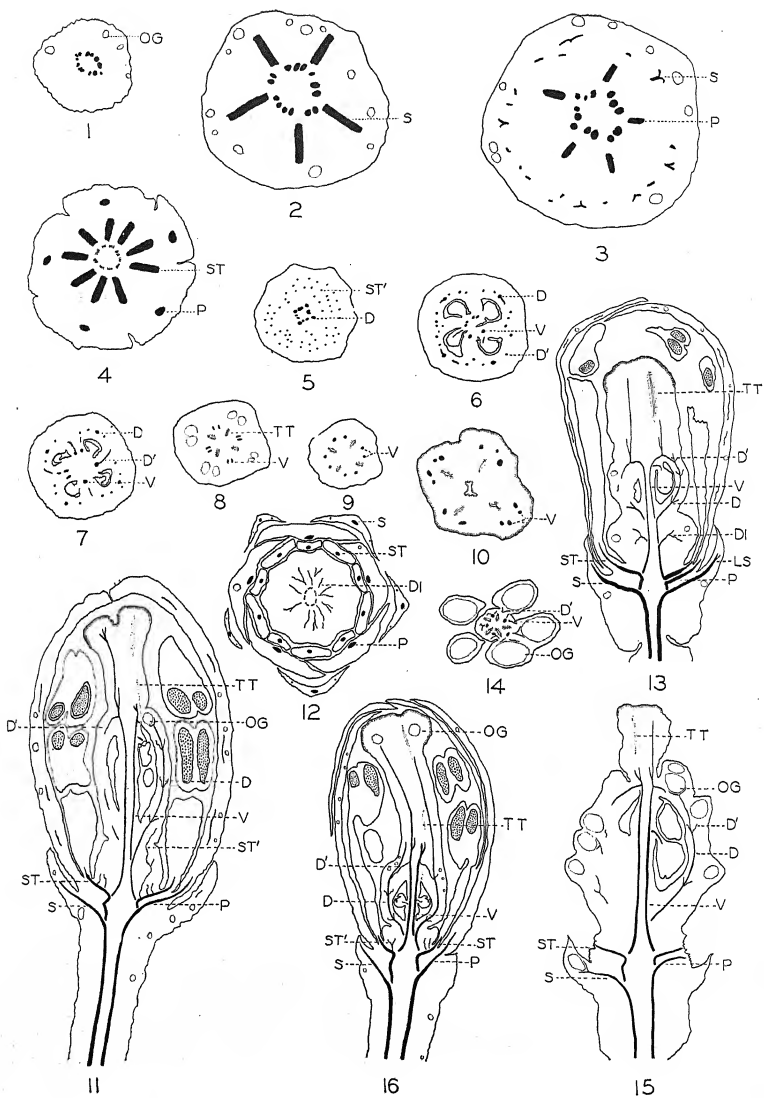
Tribe I. CLAUSENEAE:—Subtribe 1. MICROMELINAE: *Micromelum minutum* (Forst.) Wt. & Arn., *M. compressum* (Bl.) Merr., *M. falcatum* (Lour.) Tan., *M. integrum* (Buch.-Ham.) Roem., *M. hirsutum* Oliv., *M. ceylonicum* Wt., *M. scandens* Reehinger, *M. pubescens* Bl. Subtribe 2. CLAUSENINAE: *Glycosmis citrifolia* Lindl., *G. puberula* Lindl., *G. chlorosperma* Blume, *G. Greenei* Elmer, *G. pentaphylla* (Retz.) Correa, *G. Craibii* Tan., *G. macrantha* Merr., *G. cochinchinensis* Pierre, *Clausena lansium* (Lour.) Skeels, *C. anisum-olens* (Bl.) Merr., *C. anisata* Engl., *C. excavata* Burm. f., *C. grandifolia* Merr., *C. Worcesteri* Merr., *C. laxiflora*, *C. heptaphylla* (Exb.) Wt. & Arn., *C. mollis* Merr., *C. Guillauminii* Tan., *C. inaequalis* (DC.) Benth., *C. microphylla*; *Muraya paniculata* Linn., *M. crenulata* (Turcz.) Oliv., *M. Koenigii* Spreng. Subtribe 3. MERRILLINAE: *Merrillia caloxylon* (Ridl.) Swingle.

Tribe II. CITREAE:—Subtribe 1. TRIPHASINAE: *Wenzelia brevipes* Merr., *W. melanesica* Swingle;

Ozantha aurantium Tan., *O. neo-caledonica* (Guill.) Tan.; *Triphasia trifolia* (Burm. f.) P. Wils., *T. grandifolia* Merr., *T. Brassii* (Tan.) Swingle; *Luvunga philippinensis* Merr., *L. borniensis* Hochr., *L. scandens* (Roxb.) Wall., *L. villosa*, *L. sarmentosa* (Bl.) Kurz; *Paramignya Surasiana* Craib., *P. monophylla* Wt., *P. scandens* (Griff.) Craib., *P. hainanensis* Swingle, *P. longipedunculata* Merr. Subtribe 2. CITRINAE: *Severinia buxifolia* (Poir.) Tenore, *S. disticha* (Bl.) Swingle, *S. linearis* (Merr.) Swingle, *S. retusa* (Merr.) Swingle, *S. paniculata* (Warb.) Swingle, *S. hainanensis* Merr. & Chun, *S. trimera* (Oliv.) Swingle; *Pleiospermium dubium* (Bl.) Swingle, *P. alatum* (Wt. & Arn.) Swingle; *Limnocitrus littoralis* (Miq.) Swingle; *Burkillanthus malaccensis* Swingle; *Hesperethusa crenulata* (Roxb.) Roem.; *Citropsis Schneefurthii* (Engl.) Swingle & M. Kell., *C. gabonensis* (Engl.) Swingle & M. Kell., *C. angolensis* Excell., *C. mirabilis* (A. Chev.) Swingle & M. Kell., *C. latilata* subsp. *Gilletiana* Swingle & M. Kell.; *Atalantia monophylla* (Linn.) DC., *A. racemosa* Wt., *A. Wrightii* Tan.; *Fortunella japonica* (Thunb.) Swingle, *F. Hindii* (Champ.) Swingle, *F. margarita* (Lour.) Swingle; *Eremocitrus glauca* (Lindl.) Swingle; *Poncirus trifoliata* (L.) Raf.; *Clymenia polyandra* (Tan.) Swingle; *Microcitrus australasica* (F. Muell.) Swingle, *M. Garrawayi* (Bailey) Swingle; *Citrus reticulata* Bl., *C. grandis* Osbeck, *C. aurantifolia* (Christm.) Swingle, *C. limon* (Linn.) Burm. f., *C. sinensis* (Linn.) Osbeck, *C. medica* Linn., *C. macroptera* Montr., *C. ichangensis* Swingle, *C. micrantha* Wester. Subtribe 3. BALSAMOCITRINAE: *Swinglea glutinosa* Merr.; *Aegle Marmelos* (Linn.) Corr.; *Afraegle gabonensis* (Swingle) Engl., *A. paniculata* (Schum.) Engl.; *Aeglopsis Chevalieri* Swingle; *Feronia limonia* Swingle; *Feroniella lucida* (Scheff.) Swingle, *F. oblata* Swingle.

Of the 94 species studied, 26 were obtained from trees grown in the University of Maryland greenhouses and the U. S. Department of Agriculture exhibition greenhouses and from preserved material sent in by collectors. The balance were obtained and identified, through the kindness of Dr. Walter T. Swingle of the U. S. Department of Agriculture, from dried herbarium specimens loaned to him by the following herbaria: University of California, Arnold Arboretum, Gray Herbarium at Harvard University, U. S. National Herbarium, Philippine Bureau of Science, British Museum Herbarium at London, Herbarium of Botanic Gardens at Singapore, Upsala Botanical Museum, U. S. Plant Exploration & Introduction Herbarium, Kew Herbarium, Herbarium of Museum of Natural History at Paris, Botanical Museum Herbarium at Berlin-Dahlem, Rijks Herbarium at Leiden, and University of Aberdeen (Scotland). All material, both herbarium and living, was identified by Dr. Swingle.

The freshly collected buds were fixed in Nawaschin's fluid and dehydrated and imbedded by Zirkle's butyl alcohol method. The material was sectioned 20 microns thick and serial sections mounted and stained by the safranin-fast green method. Herbarium specimens had to be given a softening treatment,



slightly modified from the Juel method (1918) of softening and restoring specimens. The buds or fruits were first heated in water below 100°C. for one to two hours, then transferred to dilute ammonia (1 part ammonium hydroxide to 19 parts water) and placed in an oven at 50°C. for 12 to 24 hours. This treatment softened the flattened specimens and restored them to approximately their original size and shape. After the buds were rinsed thoroughly in running water, the same technique was followed as for fresh material. By this softening treatment sections were obtained which compared favorably with freshly fixed materials, the only apparent difference being the shrunken appearance of the protoplasts.

The drawings, some of which are semi-diagrammatic, were made with the aid of a Bausch & Lomb microprojector. The magnifications are noted in the explanations accompanying the figures.

FLORAL STRUCTURE

A brief description of the floral structure, taken in part from Engler (1931) and in part from actual observations, will aid in an understanding of the anatomical descriptions given below.

The Aurantioideae is a subfamily of the Rutaceae and is made up of trees, shrubs, and woody climbers native to tropical and subtropical Asia, eastern Australia, and Africa. The flowers are typically 3- to 5-parted, regular, mostly perfect, sometimes becoming unisexual by abortion. The sepals are free or united, often forming a bowl-shaped calyx. The petals are usually imbricate, seldom valvate, free or somewhat united. The stamens are rarely the same in number as the petals, mostly twice as many or 4-12 times more, with filaments free or partially fused. The floral axis between the stamens and the ovary is usually enlarged into a ring-, cushion-, or cup-shaped disc or into a gynophore. The ovary is made up of usually 3-5, but sometimes fewer or more numerous carpels, entirely united, mostly with 1-2, but sometimes numerous, ovules in each carpel. The fruit is a berry, chiefly with a pulp consisting of juicy emergences from the carpel walls. Oil glands are found in all parts of the flower, and frequently unusually large oil glands are found at the tips of the sepals, stamens, disc, carpels, or stigma lobes.

TRIBE CLAUSENEAE

The floral organs of the members of this tribe are characterized by the fact that the traces which supply each whorl arise independently from the axis and display no trace of fusion with each other.

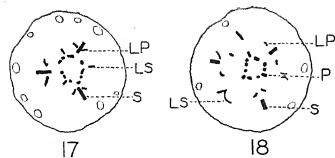
SUBTRIBE MICROMELINAE.—*Micromelum* (fig. 1-11).—The vascular tissue of the pedicel is arranged in a varying number of separate bundles. The first bundles to diverge from the axis are the five sepal midribs (*S*), which follow an outwardly ascending course through the receptacle and turn abruptly outward at the base of the sepals, which are fused into a bowl-shaped calyx. In several species lateral sepal traces (*LS*) are present; these depart from the axis at about the same or a slightly higher level than the sepal midribs and in alternation with them. The five petal midribs (*P*) next diverge in alternation with the sepal midribs and pass horizontally to the petals. The ten stamen traces (*ST*) follow soon after the petal traces, so soon in several species that the antepetalous stamen traces appear fused with the petal midribs and thus may move outward slightly ahead of the antepetalous stamen traces. The disc is cushion-shaped or stalk-like and receives from the top of the stamen traces numerous branches (*ST'*), some of which enter the ovary wall.

The five dorsal traces (*D*) of the carpels diverge on the petal radii, pass upward through the outer carpel walls, giving off several branches (*D'*), and end just below the five large oil glands (*OG*) at the top of the ovary. The vascular tissue which is left after the departure of the dorsal carpel traces forms five traces, each of which represents two fused ventral bundles (*V*) of two adjacent carpels. Each of these traces passes upward through the ovary axis, sending off branches to ovules in two adjacent carpels. At the top of the ovary each fused ventral trace splits into paired bundles, each of which is joined by one or more branches from the dorsal bundles. They then pass upward through the style, the paired bundles alternating with the stylar canals (*TT*), to the stigma. In some species, some or all of the ventral traces may remain fused for their entire length. In *M. integerrimum* each of the ventral traces ends just below a large oil gland; however, in most species of *Micromelum* these abnormally large oil glands are not present in the stigma.

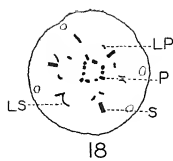
SUBTRIBE CLAUSENINAE.—Except for minor differences, the vascular plan throughout the Clauseninae is uniform and is similar to that of *Micromelum*. In *Murraya* the disc is stalk-like or cushion-shaped, while in *Glycosmis* and *Clausena* it is a short, more or less swollen gynophore.

Glycosmis (fig. 12-13).—The stamen traces do not give off branches in this genus, so that the disc receives no vascular supply except in the case of *G. cochinchinensis*, *G. chlorasperma*, and *G. citrifolia*, where several weak traces (*DI*) depart from the stele

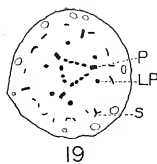
Fig. 1-11. *Micromelum scandens*; (In these and following figures: *S*, sepal midrib; *LS*, lateral sepal bundle; *P*, petal midrib; *LP*, lateral petal bundle; *ST*, stamen bundle; *ST'*, disc trace arising from stamen bundle; *DI*, disc trace arising from axis; *D*, dorsal carpel bundle; *D'*, branch of dorsal carpel bundle; *V*, ventral carpel bundle; *V'*, branch of ventral carpel bundle; *TT*, stylar canal; *OG*, large oil gland.)—Fig. 1-4. Successive cross sections through receptacle. $\times 17$.—Fig. 5. Cross sect. through disc. $\times 17$.—Fig. 6-8. Successive cross sect. through ovary. $\times 17$.—Fig. 9. Cross sect. through style. $\times 17$.—Fig. 10. Cross sect. through stigma. $\times 17$.—Fig. 11. Long. sect. of bud. $\times 10$.—Fig. 12-13. *Glycosmis citrifolia*.—Fig. 12. Cross sect. through disc. $\times 22$.—Fig. 13. Long. sect. of bud. $\times 15$.—Fig. 14. *Clausena heptaphylla*, cross sect. through top of ovary. $\times 17$.—Fig. 15. *Clausena grandifolia*, long. sect. of pistil. $\times 14$.—Fig. 16. *Murraya paniculata*, long. sect. of bud. $\times 11$.



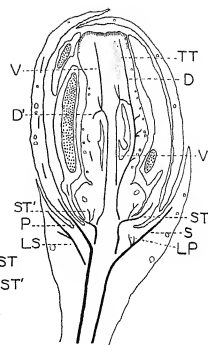
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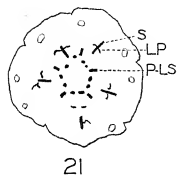
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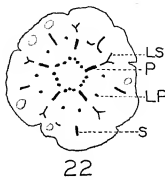
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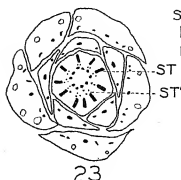
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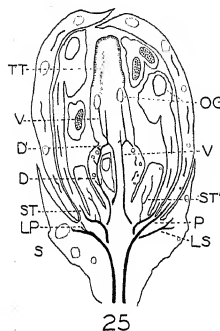
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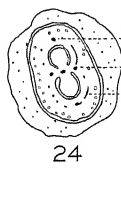
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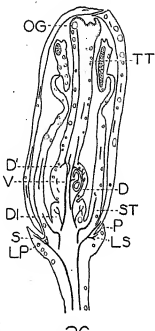
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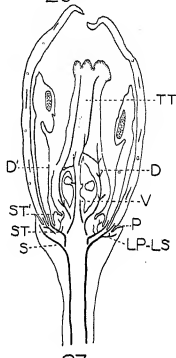
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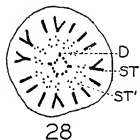
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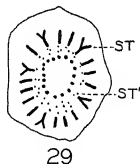
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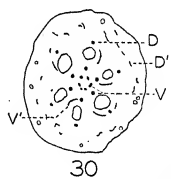
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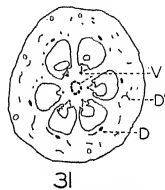
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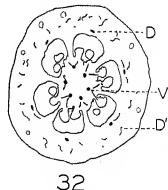
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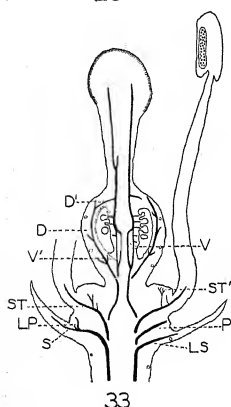
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into the disc. The dorsal carpal bundles in most species end at the top of the ovary, and in the case of *G. Craibii*, *G. chlorosperma*, and *G. Greenii* they terminate just below the large oil glands there. However, in *G. cochinchinensis* and *G. macrantha* they continue about half way up the style, opposite the styler canals. Occasionally large oil glands are present also in the stigma (*G. macrantha*, *G. pentaphylla*), in which case the ventral traces end adjacent to them.

Clausena (fig. 14-15).—Several species of *Clausena* have in the tips of the sepals large oil glands around which the sepal midribs anastomose. Since, as in *Glycosmis*, the stamen traces do not branch, no vascular tissue is present in the disc, except in *C. mollis*, where several very weak traces diverge from the axis. At the top of the ovary the dorsal carpal bundles ramify below the large oil glands which are usually present. Some of these branches fuse with the ventral bundles, and some pass a short distance up the style. In some species large oil glands are present in the stigma.

Murraya (fig. 16).—The disc, which is similar to that in *Micromelum*, derives its vascular supply from branches from the stamen traces; it receives no independent traces from the axis. In most of the species studied, the dorsal traces move up the style opposite the styler canals and end in or just below the stigma. In *M. crenulata*, however, the dorsal traces end below the large oil glands at the top of the ovary. The ventral bundles, and in *M. Koenigii* the dorsal bundles also, terminate below large oil glands in the stigma.

SUBTRIBE MERRILLIINAE.—*Merrillia*.—There is no essential difference between the vascular plan of *Merrillia* and that of the preceding groups. The disc, which is like that of *Murraya*, receives no traces either from the stamens or from the axis. The dorsal carpal bundles enter the style, but pass only a short distance upward.

TRIBE CITREAE

In the Citreae there occurs a fusion of the traces supplying the sepals and petals (with the exception of *Afraegle*, *Aeglopsis*, and *Aegle* in the subtribe Balsamocitrinae). The degree of fusion ranges from the simple union of the sepal midrib with the lateral traces of the two adjacent petals just above it, to the condition where, in addition to this type of adnation, the lateral sepal traces are fused to or arise as branches of the petal midrib just above them.

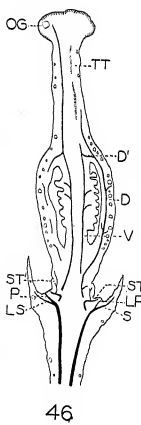
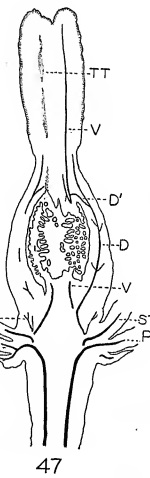
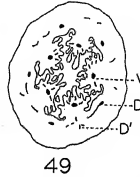
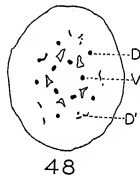
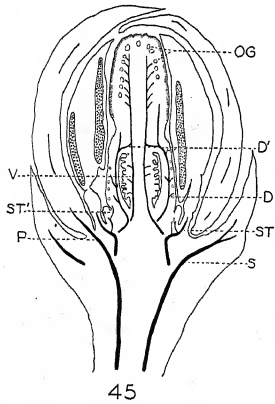
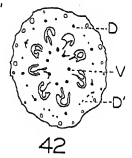
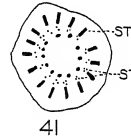
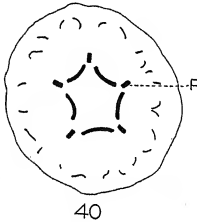
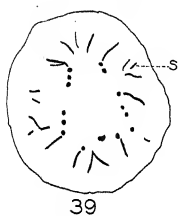
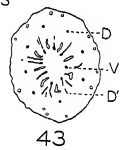
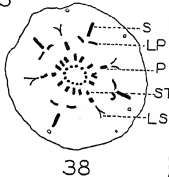
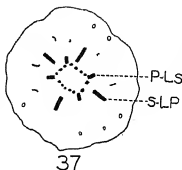
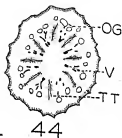
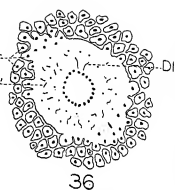
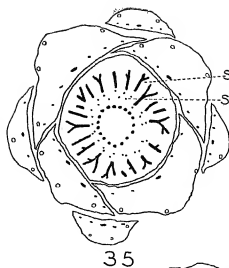
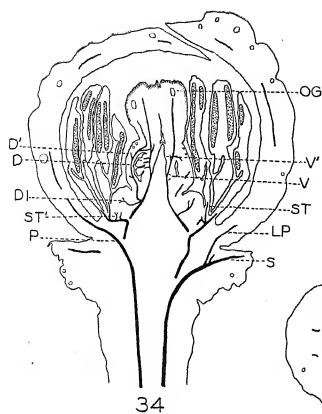
SUBTRIBE TRIPHASINAE.—In the flowers of this subtribe only the simpler type of fusion, that is, the fusion of sepal midrib and lateral petal traces, is commonly present. There are, however, some cases of slight fusion of the petal midrib with the lateral sepal bundles.

A. Wenzelia Group.—*Wenzelia*.—Each of the first five bundles to emerge from the stele represents a sepal midrib fused to the lateral petal bundles. As these bundles pass outward below the base of the petals, each gives off two branches which enter the two adjacent petals alternating with the sepal lobes. The petal midribs arise independently from the axis at a slightly higher level, alternating with the sepal midribs. Thus in contrast to the Clauseneae, where the lateral petal bundles are branches of the petal midribs, the petals receive their vascular supply from two different whorls of bundles. The ten stamen traces next leave the stele, and from the edges of their gaps arise quickly a number of traces which enter the disc and sometimes pass up into the ovary. At the base of the stalked ovary the dorsal carpal bundles move outward from the axis and run up through the carpal walls, giving off several branches. Some of the branches join the ventral bundles at the top of the ovary. The remaining vascular tissue forms the ventral bundles, which send off branches to the ovules and then, together with the dorsal bundles, pass up through the style to the stigma, where they end in large oil glands. The two ventral bundles of each carpal remain fused until they pass into the style, but at this level they, as well as some of the dorsal bundles, may split into two separate bundles. In the style the dorsal bundles occupy a position opposite the styler canals, while the paired ventral bundles alternate with the styler canals.

B. Oxanthera Group.—*Oxanthera*.—Due to the fact that there was inadequate material for a study of this genus, no details of the vascular supply will be given here. In general, however, the arrangement of the bundles seems to correspond to that of other genera of this subtribe.

C. Luvunga Group.—*Luvunga*.—The first whorl of bundles, twice as many as the calyx lobes, which diverges from the axis consists of fused sepal midrib-lateral petal bundles alternating with fused lateral sepal-petal midrib bundles. Beneath the base of the petals each sepal midrib sends off two lateral branches which enter adjacent petals. The fusion of the lateral sepal-petal midrib bundles is usually only slight and is irregular, even within a single flower bud. In most cases the lateral sepal traces diverge from the petal midrib close to the axis, or the petal midribs may merely arise from the edges of the gaps left by the lateral sepal traces. However, in *L. philippinensis* the separation occurs just before the bundles pass below the petals. In *L. villosa* the lateral petal traces may branch from the lateral sepal traces or from the sepal midribs. In some species there is an extensive anastomosis of the lateral sepal traces with the sepal

Fig. 17-20. *Triphasia trifolia*.—Fig. 17-19. Successive cross sect. through receptacle. $\times 21$.—Fig. 20. Long. sect. of bud. $\times 13$.—Fig. 21-25. *Severinia buzifolia*.—Fig. 21-22. Successive cross sect. through receptacle. $\times 15$.—Fig. 23. Cross sect. through base of disc. $\times 15$.—Fig. 24. Cross sect. through ovary and disc. $\times 15$.—Fig. 25. Long. sect. of bud. $\times 13$.—Fig. 26. *Paramignya Griffithii*, long. sect. of bud. $\times 6$.—Fig. 27. *Atalantia monophylla*, long. sect. of bud. $\times 10$.—Fig. 28. *Fortunella Hindii*, cross sect. through base of disc. $\times 9$.—Fig. 29-33. *Poncirus trifoliata*.—Fig. 29. Cross sect. through base of disc. $\times 7$.—Fig. 30-32. Successive cross sect. through ovary. $\times 7$.—Fig. 33. Long. sect. of pistil. $\times 7$.



midribs, so that it is rather difficult to ascertain from which traces the lateral petal bundles are derived. The origin of the vascular bundles entering the stamens, disc, and carpels is similar to that in *Wenzelia*. The style and stigma were missing from some ovaries, but in those which were present the dorsal and ventral bundles ended adjacent to large oil glands in the stigma.

Pararnigyna (fig. 26).—As in *Luvunga*, there is a fusion not only of the sepal midrib with the lateral petal traces, but also of the lateral sepal traces with the petal midrib. The divergence of the latter may take place close to the axis or almost beneath the petals. The disc derives its vascular supply from several bundles arising directly from the stele. The disc traces ramify about the large oil glands often present in the disc, as do also the dorsal carpal bundles which arise at a slightly higher level. The dorsal bundles do not enter the style, but ramify about the oil glands at the top of the ovary. Some of the branches from the dorsal bundles fuse with the ventral bundles, which pass up through the style in alternation with the styler canals and end close to the oil glands in the stigma, around which they may branch.

D. *Triphasia* Group.—*Triphasia* (fig. 17-20).—The bundle arrangement in *Triphasia* is similar to that in *Wenzelia*. However, in addition to the sepal midribs, there are also lateral sepal bundles arising almost at the same level as the latter, and alternating with them. They pass outward, splitting into two branches which enter adjacent sepal lobes. The vascular supply to the disc may arise either as branches of the stamen traces or from the edges of the gaps left by the stamen bundles as they diverge from the stele.

SUBTRIBE CITRINAE.—A. Primitive Citrous Group.—In this group there is a great deal of irregularity in the fusion of the lateral sepal bundles with the petal midribs.

Severinia (fig. 21-25).—In *Severinia* a fusion of sepal and petal traces similar to that in *Luvunga* occurs. However, in all the species studied, the divergence of lateral sepal traces from the petal midribs takes place close to the axis, and in one species, *S. linearis*, there is no such fusion, there being no lateral sepal bundles. The bundles of the cup-shaped disc arise as branches of the stamen traces. The dorsal bundles of the two to three carpels, after sending branches to the ventral bundles, end just below the large oil glands usually present at the top of the ovary. The fused ventral bundles separate into paired bundles and, alternating with the styler canals, pass up through the style to the stigma, where they, too, end close to large oil glands.

Pleiospermium.—In *P. alatum* the lateral petal bundles sometimes arise independently and sometimes

as branches of the sepal midribs or petal midribs. The lateral sepal bundles and the petal midrib arise from the same bundle group in the axis, but the petal midrib does not send any branches into the calyx. In *P. dubium*, however, the petal midribs diverge from the axis totally or partially fused with the lateral sepal bundles, from which they quickly separate. The saucer-shaped disc receives branches from the ten stamen traces. The dorsal bundles enter the style and pass about half way up to the stigma. The ventral bundles are joined by branches of the dorsal bundles at the top of the ovary and pass up, either as fused or paired ventral bundles, to the stigma, where they end in the large oil glands.

Burkillanthus and *Limnocitrus*.—Except for minor differences, the vascular systems of these two genera are rather like that of *Pleiospermium*.

Hesperethusa.—As in *Pleiospermium*, the amount of fusion of sepal and petal traces is irregular. The eight stamen traces are unbranched, and the disc derives its vascular supply directly from the axis. These disc bundles ramify around the oil glands in the disc. The four dorsal bundles end in oil glands at the top of the ovary, and the eight paired ventral bundles end in large oil glands in the stigma.

B. Near-Citrous Group.—Species of *Citropsis* and *Atlantia*, as a rule, exhibit no fusion of the lateral sepal bundles with the petal midribs.

Citropsis.—The first whorl of bundles to diverge from the axis consists of four fused sepal midrib-lateral petal bundles. These are closely followed by a whorl of smaller lateral sepal bundles. In contrast to the genera of the preceding group, there is no fusion whatever of the lateral sepal bundles with the four petal midribs, which arise independently. The traces to the ring-shaped disc arise as branches of the stamen bundles; in some species they may also arise at the edges of the stamen gaps or slightly higher up the axis. The dorsal carpal bundles in most species run about half way up the style, opposite the styler canals, but in *C. gabonensis* they end below the large oil glands at the top of the ovary. The four fused ventral bundles are always joined by branches of the dorsal bundles at the top of the ovary, after which they pass up the style in alternation with the styler canals, and end in the stigma close to the large oil glands about which they may ramify.

Atlantia (fig. 27).—In general, the vascular plan of this genus is the same as that of *Citropsis*. However, in *A. monophylla*, after the departure of the sepal midribs a second whorl leaves the axis; each bundle of this whorl represents a petal midrib fused with lateral sepal and lateral petal bundles. Two branches are given off by each of these fused bundles before they have passed very far outward, and each of these branches splits into a lateral petal and a lateral sepal bundle below the base of the petals.

Fig. 34-36. *Clymenia polyandra*.—Fig. 34. Long. sect. of bud. $\times 7$.—Fig. 35-36. Successive cross sect. through base of disc. $\times 9$.—Fig. 37-38. *Citrus grandis*, successive cross sect. through receptacle. $\times 9$.—Fig. 39-45. *Afraegle paniculata*.—Fig. 39-43. Successive cross sect. through receptacle and ovary. $\times 12$.—Fig. 44. Cross sect. through stigma. $\times 12$.—Fig. 45. Long. sect. of bud. $\times 10$.—Fig. 46. *Svinglea glutinosa*, long. sect. through pistil. $\times 6$.—Fig. 47-49. *Feronia limonia*.—Fig. 47. Long. sect. through pistil. $\times 7$.—Fig. 48-49. Successive cross sect. through base of ovary. $\times 18$.

C. True Citrous Group.—In this group, there is, as in Group B, no fusion of the lateral sepal traces with the petal midribs, except in the case of several species of *Citrus*. In contrast to the two preceding groups, the stamen traces are four or more times the petals in number.

Fortunella (fig. 28).—The arrangement of the vascular bundles is about the same as in *Citropsis*. The same type of fusion of sepal and petal traces occurs. There are no lateral sepal traces originating directly from the axis. The number of bundles leaving the stele to supply the 15 to 23 stamens is fewer than the number of stamens, but some of the bundles divide laterally into two traces as they pass toward the stamens. In *F. margarita* only the antepetalous stamen traces branch, but in other species either antepetalous or antesepalous stamen traces may branch. The traces to the annular disc originate from the tops of the stamen bundles. The dorsal carpel bundles in all species enter the short style, and in some species penetrate almost to the stigma. The ventral carpel bundles, as usual, end in the stigma, close to the large oil glands which are commonly present there.

Eremocitrus.—As in the other genera of this group, the first bundles to diverge from the axis are the five fused sepal midrib-lateral petal bundles, which separate into their component traces below the petal bases. These are quickly followed by five smaller, lateral sepal traces, and then four petal midribs leave the stele. As in *Fortunella*, some of the antepetalous stamen bundles divide after leaving the stele in order to supply the pleiomerous androecium. The antesepalous stamen bundles do not display any lateral branching. Branches from the tops of the stamen bundles enter the disc. Both the ventral and dorsal carpel bundles enter the style, the latter moving upward opposite the stylar canals and the former in alternation with them. The dorsal carpel bundles end about half way up the style, and the ventral bundles pass up to the stigma.

Poncirus (fig. 29-33).—The vascular supply to the calyx, corolla, androecium, and disc is the same as in *Eremocitrus*. At the base of the 6-carpellate ovary the vascular tissue remaining after the departure of the six dorsal bundles forms the six ventral bundles, which then divide radially so that two whorls of six bundles each result. Each bundle of the outer whorl takes a position opposite the two placentae of a carpel, and the bundles of the inner whorl move toward the center of the axis. The outer ventral bundles send branches (V') to the outer carpel walls and supply also the ovules in the lower part of the ovary, gradually becoming smaller as they pass upward. About half way up the ovary the inner whorl of ventral bundles moves outward to join the outer whorl and supplies the ovules in the upper half of the ovary. Instead of standing opposite the carpels, as did the bundles of the outer whorl, they alternate with them and supply the ovules of one placenta in each of two adjacent carpels, which latter condition is typical of the genera previously described. Near

the top of the ovary, branches from the dorsal carpel bundles fuse with the ventral bundles, which then pass upward, in alternation with the stylar canals, to the stigma. The dorsal bundles end at the base of the style.

Clymenia (fig. 34-36).—The vascular supply to the calyx and corolla is similar to that for *Poncirus* and *Eremocitrus*. The occurrence of approximately 100 stamens leads to a great deal more branching of the stamen bundles than has taken place in other genera with more than twice as many stamens as petals. The stamen bundles leave the axis in a single whorl, and as they approach the periphery, both the antepetalous and antesepalous stamens branch tangentially and vertically to supply the individual stamens. The filaments separate from the base of the disc in two to three irregular whorls, although the vascular tissue originates in a single whorl of bundles. The annular disc receives some branches from the stamen bundles and some traces directly from the axis. The dorsal bundles of the 17 carpels arise independently from the stele, branch repeatedly as they move upward through the carpel walls, pass up the style one opposite each stylar canal, and end a little below or in the stigma. The remaining vascular tissue at the base of the ovary forms 17 fused ventral bundles which take positions alternating with the carpels and supply placentae of adjacent carpels. About half way up the ovary, branches from the ventral bundles move to the center of the ovary, pass upward and end in a dome of densely cytoplasmic cells beneath the base of the style. At the top of the ovary the ventral bundles divide into two, fuse with branches from the dorsal carpel bundles and pass up the style, in pairs alternating with the stylar canals, to the stigma.

Microcitrus.—The calyx and corolla of *M. Garrawayi* exhibits the fusion of the sepal midribs with the lateral petal bundles typical of this group. However, in *M. australasica* there is no fusion whatever of the traces supplying the sepals and petals, the lateral petal traces being given off by the petal midribs after they have entered the petals. The vascular supply of the androecium, disc, and pistil differs little from that of *Fortunella*.

Citrus (fig. 37-38).—In *C. micrantha*, *C. ichangensis*, and *C. macroptera* of the subgenus *Papeda* the vascular supply to the calyx and corolla exhibits the same type of fusion as in *Poncirus* and *Eremocitrus*. However, in *C. reticulata*, *C. grandis*, *C. aurantifolia*, *C. limon*, and *C. sinensis* of the subgenus *EuCitrus* there is not only fusion of the sepal midrib with the lateral petal bundles, but also fusion of the lateral sepal bundles with the petal midrib. When the petal midrib reaches its position beneath the petal base, one or more branches from it continue into the base of the calyx, forming the lateral sepal bundles. The rest of the vascular system differs in no essential way from that of *Microcitrus*, *Eremocitrus*, or *Fortunella*.

SUBTRIBE BALSAMOCITRINAE.—In *Afraegle*, *Aeglopsis*, and *Aegle* the sepal and petal midribs arise independently as in the Clauseneae, but differ from them in other ways. In *Swinglea*, *Feronia*, and *Feroniella*, however, fusion of the sepal midribs with the lateral petal bundles occurs, and in *Swinglea* there is also a slight fusion of the lateral sepal traces with the petal midrib.

A. Tabog Group. — *Swinglea* (fig. 46). — As mentioned above, the vascular plan of this genus differs markedly from *Afraegle*, *Aeglopsis*, and *Aegle*. The first bundles to leave the stele are the five fused sepal midrib-lateral petal bundles. These are soon followed by a whorl of five fused lateral sepal-petal midrib bundles, which separate into their component traces shortly after leaving the stele. Some of the lateral petal bundles arise from this second whorl rather than from the sepal midribs. The traces ramifying through the cushion-shaped disc originate both from the ten stamen bundles and directly from the axis. The antesepalous stamen traces seem to diverge slightly ahead of the antepetalous traces, which supply the five shorter stamens. The eight dorsal carpel bundles, after sending off branches which fuse with the ventral bundles at the top of the ovary, pass upward and end about half way up the style. The fused ventral bundles pass up to the stigma where they ramify about the large oil glands.

B. Bael Fruit Group. — *Afraegle* (fig. 39-45). — After the departure of numerous traces to the sepals, the remaining bundles come together to form a more or less continuous vascular cylinder, which quickly assumes a pentagonal form as the five petal midribs begin to diverge. At a higher level the 15 stamen traces depart from the axis in a single whorl. From the top of the stamen bundles arise branches which ramify through the ring-shaped disc, and some of them enter the ovary wall. The dorsal carpel bundles diverge from the axis at the base of the ovary and pass upward through the carpel walls, giving off several branches. Some of the dorsal bundles end in the base of the style, while others pass upward to the base of the stigma. The balance of the vascular tissue forms into the ventral bundles which pass up through the ovary, giving off branches to the numerous ovules. At the top of the ovary, one to several branches from the dorsal bundles fuse with the ventral bundles, which then pass up through the style to the stigma. In *A. paniculata* each ventral bundle gives off branches to the series of oil glands which appear at increasing heights to the tip of the stigma. However, in *A. gabonensis* the ventral bundles pass unbranched to the large oil glands which appear only near the tip of the stigma.

Aeglopsis. — As in *Afraegle*, the bundles remaining after the sepal traces move outward form an almost continuous stele. After the departure of the petal midribs, a whorl of eight stamen traces diverges, the trace to each stamen arising independently. The dorsal bundles penetrate the style only for a very short distance, while the ventral bundles pass to the

stigma, where they end adjacent to the large oil glands there.

Aegle. — Again in this genus the bundles remaining after the divergence of the sepal midribs form a more or less continuous cylinder, which quickly assumes a square shape as the four lateral sepal bundles leave. At a somewhat higher level the five petal midribs move outward, followed by a whorl of stamen bundles, many of which, both antepetalous and antesepalous, divide laterally in order to supply the forty or more stamens. The short columnar disc receives its vascular supply both from branches from the top of the stamen bundles and from traces originating directly from the axis. As in *Afraegle* and *Aeglopsis*, the dorsal bundles enter the base of the style but do not pass upward for any great distance. A little above the base of the ovary the ventral bundles give off several weak branches which move toward the center of the axis and disappear within a short distance. In the stigma the ventral bundles break up into a number of smaller traces, each of which ends near a large oil gland.

C. Wood-Apple Group. — *Feronia* (fig. 47-49). — The five sepal midrib-lateral petal bundles are the first to depart from the stele. At a slightly higher level the petal midribs diverge independently from the axis and do not give off any branches to the calyx. The traces to the very short disc come from the gaps left in the stele by the ten stamen bundles. The five dorsal bundles disappear in the base of the style. The five ventral bundles are carried outward as the placenta separate to form a 1-loculate ovary with parietal placentae. After fusing with branches from the dorsal bundles at the top of the ovary, the fused ventral bundles separate and pass upward, the pairs alternating with the five stylar canals, to the tip of the stigma.

Feroniella. — The vascular supply to the calyx, corolla, and pistil differs little from that of *Feronia*. The stamens, four times the petals in number, are supplied by an indefinite number of bundles, some of which divide into two bundles very close to the axis. The very short disc receives several weak traces from the axis.

DISCUSSION

RELATION OF VASCULAR ANATOMY TO TAXONOMY. — From the foregoing descriptions it is seen that *Swinglea*'s tribe Clauseneae is set off clearly from the tribe Citreae by the fact that in the former group the sepal and petal midribs arise independently from the axis, while in the Citreae the sepal midribs carry out the lateral petal bundles fused to them, and in some species the lateral sepal bundles are fused in varying degrees to the petal midribs. This latter type of fusion may be very slight, the component traces of the bundle separating close to the central cylinder, or the division may take place close to or just beneath the petal bases.

Tribe Clauseneae. — Although the delimitation of the two tribes on the basis of anatomical evidence is fairly definite, the division of the tribes into subtribes and

groups is frequently not so clear. As mentioned above, the vascular systems of the genera of the tribe Clauseneae have in common the independent sepal and petal midribs. However, there is little evidence from the vascular systems which would serve as a basis for its subdivision into the three subtribes. The minor differences between the bundle arrangements are not always characteristic of a genus, much less of a subtribe, so that other methods must be used here.

Tribe Citreae.—Again in the case of the Citreae it becomes difficult to find a characteristic of the vascular plan which holds true for all the genera of a subtribe. Swingle (in press) bases his division of the tribe into three subtribes on such morphological characters as the presence or absence of pulp vesicles, hard-shelled fruit, and the number of stamens, carpels and ovules.

Subtribe Triphasiinae.—Although no characteristic of the bundle arrangement of the Triphasiinae is present which would differentiate between this subtribe and the Citrineae, the former may be divided into two groups, depending upon the type of fusion of the sepal and petal traces. *Wenzelia* and *Triphasia* have in common the absence of fusion of the lateral sepal traces to the petal midrib, which arises independently from the axis. In *Luvunga* and *Paramignya* the lateral sepal traces are carried outward by the petal midribs, a condition also found in the Citrineae-Group A. This grouping differs from that of Swingle, who, on the basis of morphological characters, places *Triphasia* and *Wenzelia* in separate groups. The similarity of *Luvunga* and *Paramignya* to Citrineae-Group A in anatomical as well as morphological characters would seem to indicate a close relationship between these two groups. The fact that the vascular plan of *Triphasia Brassii* (*Echinocitrus Brassii*) differs in no essential way from that of *Triphasia trifolia* and that of *T. grandifolia* lends support to its inclusion by Swingle as a species of *Triphasia*.

Subtribe Citrineae, Group A.—This group, which includes *Severinia*, *Pleiospermium*, *Burkillanthus*, *Limnocitrus*, and *Hesperethusa*, is clearly set off from the other two groups by the irregularity of the fusion of sepal and petal traces. In one bud of *Pleiospermium alatum*, for example, some lateral petal traces arise directly from the axis, and some arise as branches of the sepal midribs or petal midribs. On the basis of this anatomical evidence, as well as the occurrence of pulp vesicles, Swingle seems justified in removing *Pleiospermium* and *Hesperethusa* from Engler's *Hesperethusiinae* and assigning them a place close to the true citrous fruit trees. *Burkillanthus malaccensis*, which was formerly considered a species of *Citrus*, differs from the latter in having only ten stamen traces, while in *Citrus* the more numerous stamen traces divide laterally to supply the 20 to 25 stamens.

Group B. — *Citropsis* and *Atalantia*, comprising Group B, contrast sharply with Group A in not having any fusion of the lateral sepal traces with the

petal midribs. *Atalantia* differs markedly in this respect, as well as in the possession of pulp vesicles, from *Paramignya*, which exhibits fusion of the petal midribs with the lateral sepal traces. These two genera, together with *Merope*, *Severinia*, and *Pamburnus*, are considered sections of the genus *Atalantia* by Engler. However, differences in vascular anatomy, as well as morphological differences, support their recognition as separate genera.

Group C.—The outstanding difference between the genera of Group C and the preceding groups is the presence of four times as many stamens as petals. Some of the stamen traces divide laterally shortly after diverging from the axis and in *Clymenia polyantha* the stamen bundles branch both laterally and vertically to enter the approximately 100 stamens. The genera of this group, with the exception of several species of *Citrus*, differ from those of Group A in displaying no fusion of lateral sepal traces with the petal midribs. The *Citrus* subgenus *Eucitrus* is sharply set off from the subgenus *Papeda* by the fact that the species of the former group exhibit fusion both of the sepal midribs with the lateral petal bundles and of the petal midribs with the lateral sepal bundles.

Subtribe Balsamocitrinae.—The members of this subtribe, which are characterized by hard-shelled fruits, may be divided into three groups on the basis of their vascular plans. These groups correspond to the three groups proposed by Swingle (in press). In one group are *Afraegle*, *Aeglopsis*, and *Aegle*, where there is no fusion of sepal or petal traces, but the bundles remaining after the divergence of the sepal traces come together in a more or less continuous vascular cylinder, in contrast to the Clauseneae, where the bundles remain separate. *Feronia* and *Feroniella* comprise a second group, in which the lateral petal bundles arise as branches of the sepal midribs. *Swinglea glutinosa*, which is the sole member of the third group, exhibits not only fusion of the sepal midribs with the lateral petal traces, but also fusion of the petal midribs with the lateral sepal traces. The great resemblance of *Swinglea* to *Pleiospermium* and *Burkillanthus*, in the nature not only of the vascular systems but also of morphological characters, raises a question as to the relationship between these two groups,—*Balsamocitrinae* and *Citrineae, Group A*. From anatomical evidence, it seems reasonable to suppose that the *Balsamocitrinae* may represent a side line which has developed from the Clauseneae independently of the rest of the Citreae.

It is evident from the foregoing descriptions that the relationships of the Aurantioideae as shown by Engler's system of classification do not correspond to the results of this anatomical study. Although Tanaka's classification does appear to show the progressive development of this subfamily, he does not seem to be justified in setting up eight separate tribes of equal rank. In general, and with the exceptions noted above, the relationships indicated by a study of the vascular systems agree with the group-

ing proposed by Swingle (in press), who bases his taxonomic ideas on a morphological examination not only of the flowers and fruits, but also of the vegetative organs of the plants.

In table 1 is presented a summary of the types of vascular systems found in the various subdivisions of the Aurantioideae. The table is not intended to indicate a line of descent, but merely shows the trends in the vascular systems, which, like the morphological changes, may or may not be a true indication of the phylogeny of the Aurantioideae.

TABLE 1. Relation of vascular anatomy to taxonomy. S, sepal midrib independent; P, petal midrib independent; S-LP, sepal midrib and lateral petal bundles fused; P-LS, petal midrib and lateral sepal bundles fused.

	S P	S-LP P	S-LP P-LS
Tribe Clauseneae			
Subtribe Micromelinae			
<i>Micromelum</i>	×		
Subtribe Clauseninae			
<i>Glycosmis</i>	×		
<i>Clausena</i>	×		
<i>Murraya</i>	×		
Subtribe Merrillinae			
<i>Merrillia</i>	×		
Tribe Citreae			
Subtribe Triphasiinae			
A. <i>Wenzelia</i> Group			
<i>Wenzelia</i>		×	
C. <i>Luwunga</i> Group			
<i>Luwunga</i>			×
<i>Paramignya</i>			×
D. <i>Triphasia</i> Group			
<i>Triphasia</i>		×	
Subtribe Citriinae			
A. Primitive Citrous Group			
<i>Severinia</i>			×
<i>Pleiospermium</i>			×
<i>Burkillanthus</i>			×
<i>Limnocitrus</i>			×
<i>Hesperthusa</i>			×
B. Near-Citrous Group			
<i>Citropsis</i>		×	
<i>Atalantia</i>		×	
C. True Citrous Group			
<i>Fortunella</i>		×	
<i>Eremocitrus</i>		×	
<i>Poncirus</i>		×	
<i>Clymenia</i>		×	
<i>Microcitrus</i>	×	×	
<i>Citrus</i>		×	×
Subtribe Balsamocitrinae			
A. Tabog Group			
<i>Swinglea</i>			×
B. Bael Fruit Group			
<i>Afraegle</i>	×		
<i>Aeglopsis</i>	×		
<i>Aegle</i>	×		
C. Wood-Apple Group			
<i>Feronia</i>		×	
<i>Feroniella</i>		×	

OIL GLANDS.—Oil glands occur in all parts of the flower, but in most species conspicuously larger oil glands, several times the normal size, are also present at the tips of the sepals, at the top of the filaments, disc, ovary, or stigma, usually either one or two for each member of the whorl. As pointed out in the foregoing descriptions, wherever a large oil gland appears, one of the principle vascular bundles either ends close to it or sends branches about it.

ANDROECIUM.—In the more primitive *Citrus* relatives the stamens are only twice the petals in number, and the traces which supply them diverge from the stele independently and in a single whorl. In some species the antepetalous stamen traces seem to come off first, while in other species the antepetalous stamens seem to leave the axis first and may be slightly fused to the petal midribs. However, there is usually so little difference in the level of departure of the traces that it is impossible to say definitely which diverge first. It is evident, nevertheless, that the single whorl of stamens, twice the petals in number, represents two whorls which have been compressed into one, for the antepetalous stamens are usually shorter than the anteseptalous stamens, and their filaments may have a different form. Furthermore, Penzig (1887), in his study of teratological specimens of *Citrus*, reported buds in which the anteseptalous stamens were petaloid, while the antepetalous stamens were normal.

A study of the stamen traces of those species of the Citrinae and Balsamocitrinae which have three to four or more times as many stamens as petals indicates that they may have arisen by the division of members of the originally isomerous androecium, as Penzig (1887) suggested. In all species where there is pleiomery of the stamens (except in species of *Afraegle*), some of the stamen bundles branch laterally as they move outward, and in *Clymenia polyandra* there is both lateral and vertical branching of the stamen bundles to supply the numerous stamens.

Penzig's statement (1887) that in *Poncirus* only the antepetalous stamens are multiplied is borne out by anatomical studies, and in *Fortunella margarita* and *Eremocitrus* the same condition exists. However, in *Citrus* species, in confirmation of Penzig's observations, both antepetalous and anteseptalous stamens are multiplied, as is shown by the branching of the stamen traces. This condition holds true also in other genera with more than twice as many stamens as petals—*Fortunella*, *Clymenia*, *Microcitrus*, *Aegle*, and *Feroniella*.

Disc.—Moore (1936c), in a review of Saunder's paper (1934), raises the question as to the nature of the disc in the Aurantioideae. Its origin in other families has been discussed by several authors. Dawson (1936), basing her idea on the fact that the disc derives its vascular supply from the stamens, considers the basal disc in the Polemoniaceae a much reduced whorl of stamens. Lawrence (1937) regards it probable that the disc of the Boraginaceae arises from increased proliferation of the swollen base of

the ovary. Moore (1936a, 1936b), interpreting the disc in the Phascoleae as a set of undeveloped stamens, bases his conclusion on the form and position of the disc and the origin of its vascular supply from the stamen bundles.

The form of the disc in the Aurantioidae varies in the several genera. Possibly the simplest form occurs in *Glycosmis* and some species of *Clausena*, where the "disc" appears merely as a swelling of the floral axis between the androecium and gynoecium. The vascular supply consists of a varying number of small traces which arise directly from the vascular cylinder. In several genera, including *Micromelum*, *Paramignya*, *Merrillia*, *Burkillanthus*, and *Svinglea*, the disc is stalk-like and derives its vascular supply either as small traces branching from the stamen bundles or directly from the axis. In one species of *Paramignya* branches of the dorsal carpal bundles pass to the oil glands in the disc. A third type of disc is a ring in which the base of the ovary is sunken, as in *Murraya*, *Wenzelia*, *Triphasia*, *Lavanga*, *Pleiospermium*, *Linnaecitrus*, *Hesperethusa*, *Citropsis*, *Atalantia*, *Eremocitrus*, *Poncirus*, *Clymenia*, *Fortunella*, *Citrus*, *Microcitrus*, *Afraegle*, and *Aeglopsis*. In *Severinia* this latter type of disc may be extended upward to form a cup around the lower part of the ovary. Here again the disc traces may branch from the stamen bundles or come directly from the axis. In some genera—*Aegle*, *Feronia*, *Feroniella*, and some species of *Clausena*—there is little or no enlargement of the axis or constriction at the base of the ovary. In these genera the "disc" traces arise either as branches of the stamen bundles or directly from the stele.

Thus it is seen that the disc may vary in form from a swelling of the axis to a cup-like structure surrounding the base of the ovary, from which it is separated by a definite constriction. Its vascular supply is derived usually from the stamens, occasionally from the axis, or rarely from the dorsal bundles of the carpels. On the basis of this anatomical and morphological evidence it appears probable that the disc represents a whorl of modified or vestigial stamens, and not, as suggested by Penzig (1887), merely an enlargement of the floral axis between the stamens and the base of the ovary.

GYNOCIDIUM.—With one exception (*Triphasia trifolia*) the carpels are antepetalous in position whenever the gynoecium is isomerous, although where the full number of whorls is present strict alternation of the whorls should bring the carpels in line with the sepals. This agrees with the findings of a study of 33 rutaceous species, mostly in other subfamilies than the Aurantioidae, made by Saunders (1934), who states that "in the complete dicotyledon flower the uninterrupted alternation of all the whorls only occurs when some specific adjustment comes into play which provides a way of overcoming resistance to expansion, or as we may alternatively express it, of relieving the congestion which, it may be supposed, necessarily increases towards the centre as each successive whorl, still surrounded by, and continuous with, the tissue

of the earlier whorls, begins to extend outwards." She explains the antepetalous carpels of *Calodendrum capensis* by the fact that "the gynophore becomes disjoined from, and lies free within, the encircling staminal tube below the level at which the carpels begin to extend in the radial direction, so that there is no cumulative congestion effect." Although this explanation would seem plausible for such genera as *Feronia* and *Feroniella*, which have no discs, it does not explain the antepetalous carpels of such genera as *Micromelum*, *Glycosmis*, *Merrillia*, *Wenzelia*, and *Paramignya*, where the compression should be relieved by the stalk-like discs or gynophores. However, if the interpretation of the disc as a third whorl of vestigial stamens is accepted, the uninterrupted alternation of all the whorls is then seen to occur, and the antepetalous position of the carpels is explained.

STYLE.—The styler canals in the Aurantioidae are the same in number as the carpels and extend from the placentae of each carpal upward through the style to the stigma. They consist of very narrow slits bounded by a single layer of epidermal cells which are more densely cytoplasmic than the parenchyma cells surrounding them. This layer of epidermal cells is continuous from the cluster of "conducting hairs" extending from the epidermis of the placentae, which were noted in several genera by Penzig (1887), to the papillose cells of the stigmatic surface.

This condition, which was also observed in several species by Penzig, is not in agreement with Joshi's statement (1934) that "the styler canals are either continuous with the ventral traces of the carpels or make their appearance at, and occupy exactly the situation of, such traces." His conclusion that "the styler canals have been derived from, and represent modified ventral bundles of the carpels" is not in agreement with the above described observations, nor with the fact that in the Aurantioidae the ventral bundles do not disappear after supplying the ovules or merge with the styler canals, but instead continue upward through the style to the stigma either as fused or paired bundles alternating with the styler canals. The nature of the styler canal in the Aurantioidae is then in accord with our previous ideas about transmitting tissue, dating back to Capus (1878) and Gueguen (1900-1902), who showed that conduction of the pollen tube is, as a rule, effected by the inner faces of the two carpellary margins and of the placentae which are emergences from these margins.

SUMMARY

An anatomical study of 94 species comprising 20 genera of the Aurantioidae suggests the following:

In the tribe Clauseneae the sepal and petal midribs arise independently from the axis, while in the Citreae, except for *Afraegle*, *Aeglopsis*, and *Aegle*, the sepal midribs are fused to the lateral petal bundles. However, the vascular system does not offer so

definite a basis for the division of the tribes and subtribes.

The fusion of the sepal midrib and lateral petal bundles in *Pleiospermium*, *Hesperethusa*, *Luvunga*, *Triphasia*, *Wenzelia*, and *Atalantia* demonstrates their closer affinity to the Citrinae than to the Clauseneae.

Unusually large oil glands are often present at the tips of the sepals, disc, ovary, stigma, or stamens and are usually in close association with vascular tissue.

The possibility that pleiomery of the stamens arises from the division of either antepetalous stamens or both antepetalous and antepetalous stamens is indicated by branching of their traces.

The disc, which may be a swollen axis, stalk-like, ring-shaped to cup-like, or absent, appears to represent a third whorl of vestigial stamens.

The uninterrupted alternation of all the whorls is obtained, and the antepetalous position of the carpels of isomerous ovaries is explained, if the above interpretation of the nature of the disc is accepted.

The styler canals consist of narrow slits surrounded by epidermal cells and are continuous from the papillose stigmatic cells to the conducting hairs between the placentae. They are not modified ventral carpel bundles.

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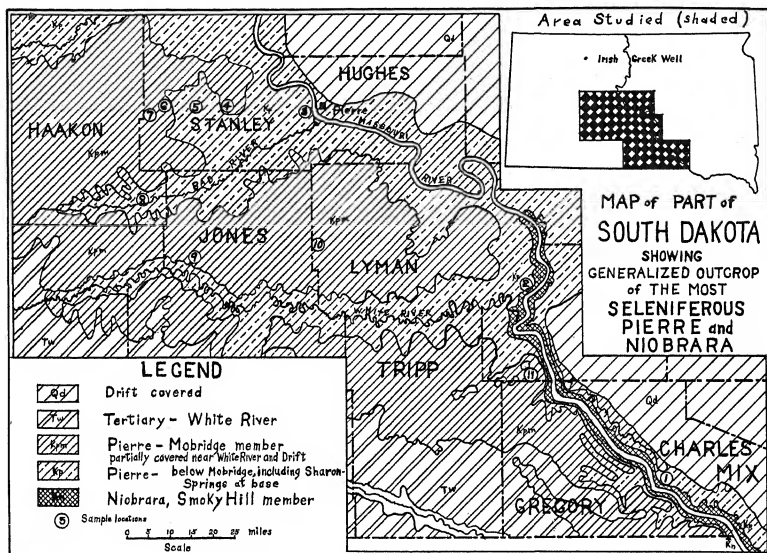
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THE STRATIGRAPHIC DISTRIBUTION OF SELENIUM IN THE CRETACEOUS FORMATIONS OF SOUTH DAKOTA AND THE SELENIUM CONTENT OF SOME ASSOCIATED VEGETATION ¹

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THE GEOLOGICAL distribution of selenium has received attention from the beginning of the studies on the selenium problem. Early in 1934 Beath et al. reported the association of selenium-bearing range plants with certain geological formations. Later in the same year Franke et al. (1934) reported that the so-called "alkali disease" of livestock in South Dakota was

associated with soils derived from Pierre shale. Beath and his coworkers have reported on extensive investigations of the geological distribution of selenium in Wyoming formations (Beath et al., 1934a, 1934b, 1935, 1937a, 1937b; Knight and Beath, 1937). Byers (1935, 1936) has reported on the selenium content of formations from various parts of the Great Plains



Map. 1.

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and has also reported on the selenium content of various zones² of the Pierre formation in South Dakota and Nebraska.

After a study of the stratigraphy of the Pierre formation (Searight, 1937) had been completed, it was possible to obtain the data presented herein on the stratigraphic distribution of selenium in the Pierre. The Pierre formation has been divided on the basis of lithology aided by micropaleontology. Late in 1936 a preliminary study on the geological distribution of selenium in South Dakota was made, the

² This division of the Pierre into zones was based on bentonite beds.

results of which have been published (Moxon, 1937). At that time the core from the Irish Creek well in the northeast corner of Ziebach county, South Dakota, was made available. This drilling penetrates Upper Cretaceous formations only; it begins possibly in the lower Fox Hills sandstone and extends down into the Greenhorn formation, as indicated by lithology and micro-fossils from washed samples. A columnar section of the formations penetrated in this drilling, ranging from Greenhorn to Pierre, is shown in chart 1.

The well core samples were supplemented by samples collected from outcroppings of the formations in numerous localities of the state identified in the field and checked by foraminifera (map 1).

The selenium contents of the samples from the well core as well as of those from the various outcroppings are given in tables 1-13.

STRATIGRAPHY.—The stratigraphy of the formations involved is discussed very briefly here, since a detailed discussion is being published elsewhere.

All strata involved are Upper Cretaceous, ranging from the Greenhorn of Benton age through the Pierre formation of Montana age. Since descriptions of the Dakota sandstone and the Graneros shale are given in many geological publications dealing with the Great Plains, they will not be given space here.

Greenhorn.—The Greenhorn formation is commonly a thin succession of calcareous, chalky, and shaly beds in the outcrops in southeastern South Dakota, around the Black Hills, and along the Rocky Mountain front. The Irish Creek well penetrated a total of 336 feet of beds assigned to the Greenhorn formation on the basis of macro- and micropaleontology. This determination is in agreement with that made in two other wells in Meade and Ziebach counties of South Dakota (Applin, 1933).

Carlile.—The Carlile formation consists of medium to dark grey non-calcareous shale, which is slightly sandy in places. Microfossils consist of characteristic agglutinated foraminifera, mostly of the genus *Haplophragmoides*. The Carlile is 384 feet thick in the Irish Creek core. Outcrops of this formation in South Dakota are limited to the southeastern part of the state and the area around the Black Hills.

Niobrara.—Beds of the Niobrara formation are made up of chalk, marl, and shale in the outcrops. A lower member of this formation, the Fort Hayes limestone, characterized by a diagnostic microfauna (Loettler, 1937), outcrops in various localities from Kansas to eastern South Dakota and has been identified in many places in Clay, Bon Homme, Turner, Hutchinson, and Davison counties in South Dakota. The Fort Hayes member probably includes approximately the lower 40 feet of the Niobrara in the well core. A thick upper member of the Niobrara, the Smoky Hill, has been identified widely both in the Black Hills area and in southeastern South Dakota by characteristic lithology and microfossils. The thickness of this member ranges from 160 to 170 feet in southeastern South Dakota outcrops to 270 feet in the well core. It is the view of the authors after

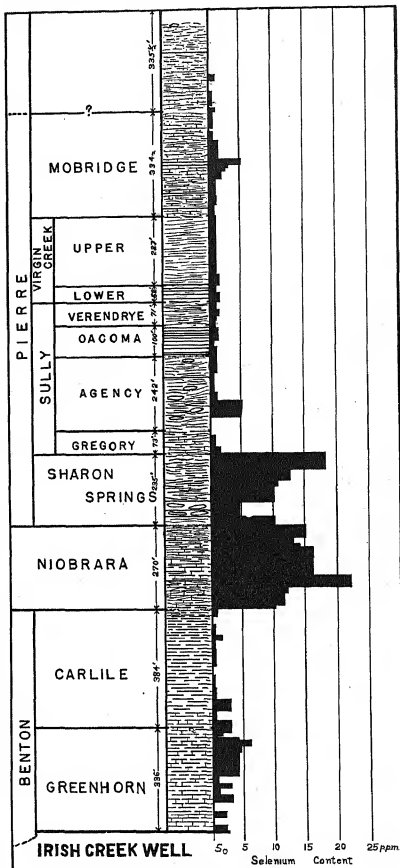


Chart 1. Position and selenium content of formations.

restudy of the well core that 95 feet of beds previously assigned to the Niobrara are Pierre in age (Russel, 1925).

Pierre.—The Pierre formation is the thickest Cretaceous formation in South Dakota. It is the parent material of the soils producing the most toxic (seleniumiferous) vegetation except in limited toxic areas where the soil has been derived from the Niobrara formation. The soils of about one-third of the area of South Dakota west of the Missouri River are derived from the Pierre formation.

Beds of the Pierre consist mostly of shale, although chalky beds of calcareous shale and marl occur. Groups of thin layers of bentonite at various stratigraphic levels, and concretions of various kinds of material at various positions, are characteristic of the formation.

On the basis of lithologic differences and microfossil content Searight (1937) has divided the Pierre formation into five members. This division, with later modifications by Searight, is as follows:

Elk Butte
Mobridge
Virgin Creek
Sully
Verendrye beds
Oacoma zone
Agency shale
Gregory marl
Sharon Springs

Although these subdivisions of the Pierre were made along the Missouri River in South Dakota, the same subdivisions are known to occur with some modification far beyond this area, into Montana, North Dakota, and Nebraska.

The beds of the Irish Creek well core between 1445 and 1680 feet in depth (chart 1) correlated by Russel (1925) have been referred to the Sharon Springs member of the Pierre along with the 10 feet immediately overlying them. Thus the Sharon Springs is 245 feet thick under northeastern Ziebach county, which is approximately 100 feet thicker than the thickest known exposure along the Missouri River.

The Sully member of the Pierre, as shown on chart 1, is made up of the Gregory marl, Agency shale, the Oacoma zone, and the Verendrye beds. The Gregory (Upper Gregory as originally defined by Searight, 1937) occupies the position between 1372 feet and 1445 feet in the Irish Creek core. The com-

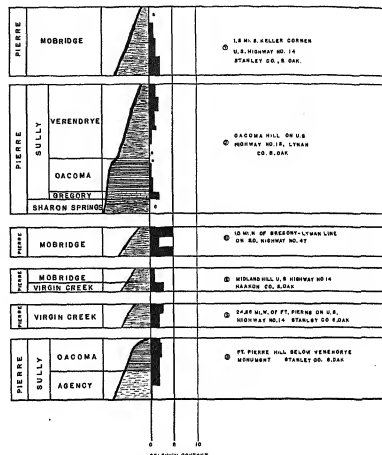


TABLE 1. *Selenium content of core samples of Irish Creek well, S.E. ¼ sec. 17, T. 15 N., R. 20 E., Ziebach county, South Dakota.*

	Selenium p.p.m.	Depth, feet	
		From	To
No core taken		0	80
Unassigned, possibly Pierre			
	0.0	80	125
	0.0	125	170
	0.0	170	215
	0.6	215	239
	*	239	270
	0.5	270	316
	0.0	316	329½
	0.8	329½	335½
Pierre Formation			
Mobridge Member	0.5	335½	393
	0.0	393	400
	0.6	400	427
	1.4	427	491
	5.0	491	500
	3.0	500	522
	2.5	522	531
	1.1	531	557
	0.8	557	586
	1.0	586	612
	1.2	612	642
	0.6	642	670
Virgin Creek, Upper			
	1.0	670	864
	1.5	864	897
Lower, top at 879 feet	1.0	897	921
	1.5	921	959
Sully Member			
Verendrye zone	1.0	959	979
	1.3	979	998
base at 1030 feet	1.0	998	1017
	0.8	1017	1042
Oacoma zone			
top at 1030 feet	1.2	1042	1076
	0.5	1076	1101
	1.0	1101	1130
Agency zone			
	1.0	1130	1186
	0.7	1186	1247
	1.3	1247	1274
	5.0	1274	1331
	0.0	1331	1372
Gregory zone			
base at 1435 feet	0.0	1372	1388
	0.7	1388	1423
	1.5	1423	1445
Sharon Springs Member	18.0	1445	1502
	12.5	1502	1535
	10.5	1535	1554
	10.0	1554	1605
	4.5	1605	1647
	10.0	1647	1680
Niobrara Formation			
	15.0	1680	1720
	13.0	1720	1742
	14.0	1742	1753
	16.0	1753	1842
	22.0	1842	1878
Bentonite	113.0	1878	1900
	12.0	1878	1900
	11.5	1900	1939
	10.0	1939	1950

TABLE 1.—Cont'd.

	Selenium p.p.m.	Depth, feet	
		From	To
Carlile Formation	0.7	1950	1975
	0.0	1975	2000
	0.5	2000	2035
	1.5	2035	2050
	0.4	2050	2081
	0.5	2081	2134
	0.0	2134	2165
	0.4	2165	2204
	0.5	2204	2243
	3.0	2243	2278
	0.5	2278	2310
	3.0	2310	2315
	3.0	2315	2334
Greenhorn Formation			
	2.7	2334	2349
	1.5	2349	2363
	*	2363	2365
	4.0	2365	2375
	6.0	2375	2393
	4.3	2393	2416
	3.5	2416	2492
	0.8	2492	2515
	2.8	2515	2529
	1.0	2529	2557
	3.0	2557	2572
	0.0	2572	2608
	1.88	2608	2625
	0.0	2625	2649
	2.2	2649	2670
	2.5	2670	2680

* No analysis.

ous sample of argillaceous limestone from the Graneros, collected in Fall River county, South Dakota, also contained no selenium (table 2, no. 3) and two (table 2, nos. 1, 2) from Shannon county are reported by Byers (1936) to contain 0.7 and 1.5 p.p.m. selenium. Samples of Graneros shale collected in Nebraska, Montana, and Wyoming contained 0.5, 0.5, and 1.0 p.p.m. selenium. Thus, the analyses from various places, in several states, indicate a range from no selenium to a maximum of 1.5 p.p.m. in the Graneros.

Greenhorn formation.—The Greenhorn formation appears to be somewhat higher in selenium content than the Graneros, but is for the most part low. The samples taken from the core of the Irish Creek well (table 1) range from no selenium up to a maximum of 6.0 p.p.m. Among 15 analyses, two contained no selenium and six contained less than 2.0 p.p.m. Only three samples contained 4.0 p.p.m. or more selenium. The stratigraphic distribution of the various amounts of selenium is indicated in chart 1, and the analyses are included in table 1.

Carlile formation.—The selenium content of the Carlile formation apparently is low. The maximum determination by analysis (3.0 p.p.m.) is only half that of the maximum of the Greenhorn. Apparently, however, selenium is commonly present in small quantities, as only two of 13 analyses from the Irish Creek

TABLE 2. *Selenium in the Graneros Formation.*

No.	Lab.No.	Authority ^a	Description	Selenium p.p.m.
1	B15157	B530	Shannon county, South Dakota	0.7
2	B15155	B530	Shannon county, South Dakota	1.5
3	60	M311	Fall River county, South Dakota	0.0
4	B14672	B530	Dawes county, Nebraska	0.5
5	B6317	B530	Weston county, Wyoming	1.0
6	B6295	B482	Carter county, Montana	0.5

^a Authorities in all tables:

B530, Byers, H. G., U. S. Dept. Ag. Tech. Bull. 530.
 B482, Byers, H. G., U. S. Dept. Ag. Tech. Bull. 482.
 M311, Moxon, A. L., S. D. Ag. Exp. Sta. Bull. 311.

well were selenium-free (table 1). All but four, however, contained less than 1.0 p.p.m. These results are in accord with those on the Carlile shale elsewhere, since a sample from Dawes county, Nebraska, cited by Byers (1936) contained 0.5 p.p.m., and the same authority cites two analyses from Trego county, Kansas, which contained 0.7 and 0.5 p.p.m. (table 3, nos. 2 and 3).

TABLE 3. *Selenium in the Carlile Formation.*

No.	Lab.No.	Authority	Description	Selenium p.p.m.
1	B14673	B530	Dawes county, Nebraska	0.5
2	B17166	B530	Upper Carlile, Trego county, Kansas	0.7
3	B17177	B530	Upper Carlile, Trego county, Kansas	0.5

Niobrara formation.—Variation in selenium content of the members of the Niobrara is great, the Fort Hays limestone being low in selenium content, whereas high concentrations commonly occur in the Smoky Hill member. As the Fort Hays member has not been definitely identified in the core of the Irish Creek well, samples were collected from the outcrop. These, although collected at various positions in the member, and over considerable vertical intervals, were uniformly low in selenium content. Of six such samples, two contained no selenium, one 0.3 p.p.m., and the remaining three from 1.0 p.p.m. to a maximum of 3.0 p.p.m. (table 4, nos. 1–6, inclusive). These data are in accord with those of Byers (1936), who reported that soils derived from the Fort Hays apparently are low in selenium. He found only six out of 64 samples of Fort Hays and Carlile from Ellis county, Kansas, to contain more than 0.5 p.p.m. In Trego county, Kansas, two samples yielded 0.5 and 0.3

TABLE 4. *Selenium in the Fort Hays member of the Niobrara Formation.*

No.	Lab.No.	Authority	Description	Selenium p.p.m.
1	1281		Composite, upper 5 feet of member. Lake Henry Spillway, Scotland, Bon Homme county, S. Dak.	3.0
2	1287		10 foot composite, Quarry, Spirit Mound, Clay Co., South Dakota	1.0
3	1290		10 foot composite, Spirit Mound above quarry, Clay county, S. Dak.	1.5
4	1291		Composite, 8–10 feet of beds below uppermost 5 feet (No. 1) Spillway, Lake Henry, Scotland, South Dakota	0.3
5	1295		Basal Fort Hays, 10 foot composite N. W. $\frac{1}{4}$ sec. 9, T. 93 N., R. 52 W., Clay county, S. Dak.	0.0
6	1297		N. E. $\frac{1}{4}$, N. E. $\frac{1}{4}$, sec. 12, T. 94 N., R. 54 W., Yankton county, S. Dak.	0.0
7	B16718	B530	Trego Co., Kansas	0.5
8	B17128	B530	Trego Co., Kansas	0.3
9	B17434	B530	Rooks Co., Kansas	6.0

p.p.m. One sample from Rooks county, Kansas, contained 6.0 p.p.m. These analyses are cited in table 4, nos. 7–9, inclusive.

As stated, the Smoky Hill member of the Niobrara commonly contains much selenium and many analyses are available. Eight samples, of which at least the upper six are Smoky Hill, covering 270 feet of Niobrara, were analyzed from the Irish Creek core. The minimum of these was 12.0 p.p.m., and the maximum was 22.0 p.p.m. This set is notably higher than that of the Smoky Hill at Yankton, as indicated by a set of analyses made from 10-foot composites over a 90-foot interval, including uppermost Niobrara (chart 2 and table 5). The minimum here was 2.0 p.p.m., and the maximum was only 16.0 p.p.m. Five of the nine samples were 5.0 p.p.m. or less, and only two were above 10.0 p.p.m. The average analysis, which in this set of carefully composited samples approximates the average selenium content, is 6.5 p.p.m. Two composite samplings of the Upper Smoky Hill at Oacoma, South Dakota, in Lyman county indicate by analysis a selenium content of 24.0 p.p.m. and 28.0 p.p.m. respectively. A number of analyses of a succession of Smoky Hill beds in Custer county, South Dakota, are listed by Byers (1935). These are highly seleniferous, since the series of 12 analyses includes only four (the lowest containing 6.0 p.p.m.) below 10.0 p.p.m. Six were 20.0 p.p.m. or more, and two of these contained 30.0 p.p.m. Two determinations of selenium in basal Smoky Hill were made from

TABLE 5. *Selenium in the Smoky Hill member of the Niobrara Formation.*

No.	Lab. No.	Authority	Description	Selenium p.p.m.
1Y	47	M311	Abandoned cement plant, N.E. ¼ sec. 17, T. 93 N., R. 56 W., Yankton county, South Dakota	3.0
2Y	48			7.0
3Y	49			9.0
4Y	50			11.0
5Y	51		Numbers 1Y to 9Y inclusive are 10 foot composites in stratigraphic order with	16.0
6Y	52		9Y at base, 1Y at top	5.0
7Y	53			3.0
8Y	54			2.0
9Y	55			2.5
Feet				
1C	B3082	B482	3 Sec. 23, T. 3 S., R. 8 E., Custer county, South Dakota	12.0
2C	B3081		3	6.0
3C	B3080		3	8.0
4C	B3079		3 Numbers 1C to 12C inclusive are samples arranged in stratigraphic order with 12C at base, 1C at top	8.0
5C	B3078		6	20.0
6C	B3077a		3	30.0
7C	B3077		1	25.0
8C	B3076		4	20.0
9C	B3075		2	30.0
10C	B3074		2	8.0
11C	B3073		2	28.0
12C	B3072		2	16.0
1M	1288		Lower Smoky Hill, composite of 10-12 feet, N.E. ¼, N.E. ¼, sec. 6, T. 94 N., R. 53 W., Yankton county, S. Dak.	3.5
2M	1289		Basal Smoky Hill, Spillway Lake Henry, Bon Homme Co., S. Dak., composite of 5 feet	6.5
3M	1272		Uppermost Smoky Hill, in contact with Sharon Springs, center Sec. 23, T. 95 N., R. 58 W.	1.3
4M	1272		Upper, white, N.E. ¼, N.E. ¼, sec. 9, T. 94 N., R. 35 W.	4.0
1L		(2) ^a	Upper Smoky Hill, 10 foot composite, upper part of outcrop, Railroad cut, Oacoma, S. Dak.	24.0
2L		(2) ^a	Upper Smoky Hill, 15 foot composite from tract level up, Railroad cut, Oacoma, S. Dak.	28.0
5K	B16184	B530	Logan Co., Kansas	16.0
6K	B16191	B530	Logan Co., Kansas	8.0
7K	B16696A	B530	Logan Co., Kansas	22.0
8K	B16892	B530	Logan Co., Kansas	18.0
9K	B16697	B530	Grove Co., Kansas	18.0
10K	B17367	B530	Rooks Co., Kansas	8.0

^a Numbers in bold face type in parenthesis (2) in this and following tables indicate locations on Map No. 1.

samples in eastern South Dakota. These were 3.5 p.p.m. and 6.5 p.p.m. Two in the upper part of the member from the same general region contained 1.3 p.p.m. and 4.0 p.p.m. The Smoky Hill is likewise highly seleniferous elsewhere, as indicated by analyses cited by Byers (1936) from Logan, Grove, and Rooks counties, Kansas (table 5). The Niobrara, particularly that equivalent to the Lower Smoky Hill, is notoriously toxic in Wyoming.

Pierre formation.—Beds of the Pierre formation contain selenium in most places in South Dakota and at most positions. Selenium content from place to place and at different positions within the formation is variable, however, to a marked degree. The Sully and Virgin Creek members apparently contain selenium only in small amounts. Although adequate data are lacking, the Elk Butte member also appears to contain low concentrations of selenium in South Dakota. The Sharon Springs member, like the underlying Niobrara, appears commonly to be rich in selenium. The Mobridge member is locally high in selenium but low, in part at least, in other localities.

TABLE 6. *Selenium content of the Sharon Springs member of the Pierre Formation.*

No.	Lab. No.	Authority	Description	Selenium p.p.m.
1		M311	Abandoned cement plant, N.E. ¼, sec. 17, T. 93 N., R. 56 W., Yankton Co., S. Dak.	45.0
2		(1)	Composite, upper 10 feet, south end Rosebud bridge, Gregory county, S. Dak.	21.0
3		(1)	Composite, lower 11 feet, south end Rosebud bridge, Gregory county, S. Dak.	16.0
4	1869	(2)	Composite, upper 15 feet, Oacoma Hill, Lyman Co., S. Dak.	0.0
5C	B3071	B482	1 ft. Numbers 5C to 10C inclusive are a stratigraphic series covering intervals as indicated. Sec. 23, T. 3 S., R. 8 E., Custer county, S. Dak.	5.0
6C	B3070		2 in.	2.0
7C	B3060		18 in.	8.0
8C	B3068		¼ in.	5.0
9C	B3067		2 ft.	7.0
10C	B3066		10 in.	2.0
11	B16176	B530	Logan Co., Kansas	10.0
12	B16177	B530	Logan Co., Kansas	22.0

The selenium content of the Sully and Virgin Creek members as determined from samples taken from the Irish Creek well core and from the outcrops is indicated in tables 1, 7, 8, 9, 10, and 11. The data show that beds of these members are rarely selenium-free. On the other hand, selenium content in excess of 3.0 p.p.m. is very uncommon—notable exceptions, however, being the Agency shale of the Sully between depths 1274 and 1331 with 5.0 p.p.m. and a hand sample of the Oacoma zone of the Sully at Rosebud

TABLE 7. *Selenium in the Gregory zone, Sully member of the Pierre Formation.*

No.	Lab.No.	Authority	Description	Selenium p.p.m.
1	1243		Center sec. 35, T. 95N., R. 54 W., Yankton Co., South Dakota	0.0
2	1866	(2)	Oacoma Hill, Lyman county, S. Dak. 6 ft. composites	2.2
3		(1)	Rosebud Bridge, Gregory Co., S. Dak.	1.7
4	36	M311	14 miles east of Statehouse, Hughes county, South Dakota	0.5

TABLE 8. *Selenium in the Agency shale, Sully member of the Pierre Formation.*

No.	Lab.No.	Description	Selenium p.p.m.	Feet
1	1887	(3) Samples from Railway cut, Ft. Pierre, Stanley county, South Dakota. Composites of thicknesses as indicated	2.0	15-30
2	1864		0.3	0-15

TABLE 9. *Selenium in the Oacoma zone, Sully member of the Pierre Formation.*

No.	Lab. No.	Authority	Description	Selenium p.p.m.
1T	1886	(3)	Feet 30-35 Samples numbered 1T to 3T inclusive are a stratigraphic series, each a composite of beds as indicated. 1T at top, 3T at base	0.0
2T	1871	(3)	15-30	0.4
3T	1882	(3)	0-15	0.7
1P	1889	(3)	30-35 Samples numbered 1P to 3P inclusive are a stratigraphic series, each a composite of beds as indicated. 1P at top, 3P at base. Ft. Pierre, below Verdrye monument	2.2
2P	1867	(3)	15-30	2.5
3P	1865	(3)	0-15	
1M		(1)	South end Rosebud Bridge, 25 foot composite, including some Agency	0.5
2M	43	M311	Rosebud Bridge, Gregory county, South Dakota	2.0
3M	33	M311	Gregory county, S. Dak.	3.0
4M	35	M311	Gregory county, S. Dak.	5.0
5M	34	M311	Lyman county, S. Dak.	Tr.

Bridge, Gregory county, South Dakota, with 6.0 p.p.m. In the Virgin Creek, a chalky zone near the top of the member has been found to be highly seleniferous (unpublished data).

The Sharon Springs member of the Pierre appears to be persistently selenium-rich, with but few exceptions. It is entirely possible that the lower part of the member is everywhere seleniferous to a high degree. Six samples were taken from the core of the Irish Creek well (table 1). One of these contained 4.5 p.p.m. of selenium, and the rest contained more than 10.0 p.p.m. The highest concentration was 18.0 p.p.m. selenium. The Sharon Springs at Yankton contained 45.0 p.p.m. A number of samples collected from a succession in Custer county, South Dakota, presumably from the base of the Sharon Springs are reported by Byers (1935) (table 6) to contain considerably less selenium than those from the Irish Creek well core and from the Yankton outcrop. The minimum selenium content here is 2.0 p.p.m. and the maximum 8.0 p.p.m., with four out of six analyses

TABLE 10. *Selenium content of the Verdrye zone, Sully member of the Pierre Formation.*

No.	Lab. No.	Description	Selenium p.p.m.
		Feet	
1T	1868	(1) 65-78	1.3
2T	1885	(1) 50-65	2.0
3T	1881	(1) 35-50	1.0
4T	1870	(1) 30-35	1.5
5T	1883	(1) 15-30	0.5
6T	1884	(1) 0-15	0.0
7	(1)	Sec. 10, T. 96 N., R. 67 W., Charles Mix Co., S. Dak. 25 foot composite	0.0

TABLE 11. *Selenium in the Virgin Creek member of the Pierre Formation.*

No.	Lab. No.	Description	Selenium p.p.m.
1		(1) Roadcut, sec. 10, T. 96 N., R. 67 W., Charles Mix county, S. Dak. 10 foot composite	3.5
2S	1873	(5) 24.85 miles west of Ft. Pierre on U.S. Highway No. 14, 10 foot composites of beds lying above 3S, lower Virgin Creek	3.0
3S	1888	(5) 24.85 miles west of Ft. Pierre on U.S. Highway No. 14, 15 foot composite of beds lying below 2S, lower Virgin Creek	2.0
4	1894	(8) Midland Hill, north of Midland, Haakon county, South Dakota, composite of uppermost 10 feet of the member	3.0

showing 5.0 p.p.m. or more. Two determinations (table 6) by the same authority from Logan county, Kansas, were 10.0 and 22.0 p.p.m. selenium.

TABLE 12. *Selenium in the Mobridge member of the Pierre Formation.*

No.	Lab.No.	Authority	Description	Selenium p.p.m.
			Feet	
1H		(1)	75-90 High Bare Hill, sec. 3, T. 96 N., R. 67 W.; Samples 1H to 6H are composites of beds as indicated, in stratigraphic order, with 1H at the top, 6H at the base	14.0
2H		(1)	65-75	22.0
3H		(1)	45-60	23.0
4H		(1)	30-45	22.0
5H		(1)	15-30	20.0
6H		(1)	0-15	10.0
1N	B3330T	B482	4 feet Samples 1N to 27N are a stratigraphic succession taken from beds of indicated thickness; 1N at top, 27N at base.	22.0
2N	B3330B	B482		20.0
3N	B3329	B482	1 in.	22.0
4N	B3328	B482		5.0
5N	B3328T	B482		32.0
6N	B3328M	B482	6 ft. 8 in.	20.0
7N	B3328B	B482	Sec. 30, T. 33 N., R. 11 W., Boyd Co., Nebraska	32.0
8N	B3327	B482	10 ft. 1 in.	1.5
9N	B3326	B482	1-2 in.	9.0
10N	B3325	B482	2 feet	8.0
11N	B3324	B482	3 feet	5.0
12N	B3322	B482	¾ in.	76.0
13N	B3321	B482	2 feet	103.0
14N	B3320	B482	1 foot	12.0
15N	B3319	B482	1 foot 3 in.	46.0
16N	B3318	B482	8-10 in.	10.0
17N	B3317	B482	1 in.	26.0
18N	B3316	B482	1 foot	50.0
19N	B3315	B482	3 feet 5 in.	29.0
20N	B3316T	B482		8.0
21N	B3314M	B482	3 ft. 4 in.	20.0
22N	B3314B	B482		26.0
23N	B3313	B482	3 ft. 3 in.	24.0
24N	B3312	B482	2 ft. 8 in.	28.0
25N	B3311	B482	2 ft. 8 in.	18.0
26N	B3310	B482	1 ft. 3 in.	20.0
27N	B3309	B482	1 foot	3.0
1K	1892	(7)	55-70 Samples 1K to 4K are composites of beds as indicated, in stratigraphic succession, with 1K at top, 4K at base. Taken 1.8 miles south of Junction of U. S. Highway 14 and S. D. Highway 63, on U. S. 14, Stanley county, South Dakota	0.0
2K	1891	(7)	40-55	1.0
3K	1876	(7)	20-40	1.3
4K	1878	(7)	0-20	2.3
1M			Resettlement purchase project	29.0

TABLE 12.—Continued.

No.	Lab.No.	Authority	Description	Selenium p.p.m.
2M ^a	B22584		Resettlement purchase project, 2 feet below surface, sec. 2, T. 107 N., R. 73 W., Lyman county, South Dakota	12.0
3M	1890	(4)	Composite of 17 feet of beds, 16.2 miles west of Ft. Pierre, South Dakota	3.0
4M	1893	(8)	Hill north of Midland, U. S. Highway No. 14, composite of 15 feet of beds	1.0
5M	1880	(9)	Roadcut, 8 miles east of Stamford on U. S. 16, composite of 5 feet of beds	1.5
6M ^B	1895	(10)	½ mile west of Junction, U. S. Highways 16 and 83, on Highway 16, 10 foot composite	3.0
7M ^a	B22581		6 miles east of Cottonwood, well below top of the member	0.8
8M ^a	B22582		7.1 miles west, 5.2 miles north of Kadoka, South Dakota	0.4
9M ^a	B6850		Yellow shale, 2 miles north of Promise, Dewey county, South Dakota	4.0
10M ^a	B22580		South line, sec. 29, T. 15 N., R. 2 E., Harding county, South Dakota	0.2
12M ^a	B22579		Southwestern North Dakota, 14 miles south of Marmarth, North Dakota, on North Dakota Highway No. 12	0.2

^a Analyses by Division of Soil Chemistry and Physics; Courtesy H. G. Byers, Chief; samples collected by Searight.

The Mobridge member of the Pierre commonly contains selenium. Variation, however, is great, a range between 0.0 p.p.m. and 103.0 p.p.m. being known. Variation in selenium content of this member from area to area appears to occur. A complete set of samples from base to top, consisting of 15 foot composites collected in Sec. 3 T. 97 N., R. 67 W., showed selenium content as indicated in table 12, nos. 1H-6H. In the set the minimum selenium content was 10.0 p.p.m. in the basal 15 feet of beds, and the maximum was 23.0 p.p.m. Since all samples are composited, the average selenium content is 18.5 p.p.m. On the other hand, 12 determinations from the Irish Creek core show a minimum of 0.0 p.p.m. and a maximum of only 5.0 p.p.m. (table 1). A set of four composite samples collected 1.8 miles south of the Junction on Federal Highway 14 and South Dakota Highway 63, along U. S. 14, covering 70 feet of Mobridge, shows a minimum of 0.0 p.p.m. and a maximum of only 2.3 p.p.m. Other analyses of Mobridge samples are cited in table 12. An important group of results is cited by Byers (table 12, nos. 1N-27N, inclusive). These show important bed to bed

variation. Of 27 analyses, only eight contained 10.0 p.p.m. or less selenium, 17 contained 20.0 p.p.m. or more, three contained 50.0 p.p.m. or more, two, 75.0 p.p.m. or more and one sample contained more than 100.0 p.p.m. The average selenium content of the beds at this place is thus extremely high.

The data indicate that in some areas the Mobridge is highly seleniferous, whereas in other localities the selenium content is no higher than that of the Sully and Virgin Creek.

The writers would call attention to the association of high concentrations of selenium with the Mobridge where it is thin, and the low selenium content in these beds in localities where the thickness of the member is greatly expanded. This association suggests that rate of deposition of the sediments containing selenium may be an important factor in selenium distribution and thus also be a determining factor in the distribution and extent of toxic seleniferous soil areas.

The authors have observed that some of the most toxic (seleniferous) farms are located on soils derived, in part at least, from the Mobridge member of the Pierre formation. The Brozik farm (Sec. 21, T. 100 N., R. 72 W., Gregory county, S. Dak.) and the Dean farm (Sec. 28, T. 3 N., R. 31 E., Stanley county, S. Dak.) both have a reputation of being very toxic and are both located on soils derived primarily from Mobridge.

Analyses of samples collected from the Elk Butte member of the Pierre in South Dakota indicate the presence of selenium in these beds. Those available, as cited in table 13, are low, showing 1 p.p.m. or less

TABLE 13. Selenium content of the Elk Butte member of the Pierre Formation.

Lab. No.	Feet	Location and description	Selenium p.p.m.
G573	0-10	Composite samples as indicated collected 2½ to 3 miles north of Wewela, South Dakota, in stratigraphic order, listed beginning at base of section	0.6
G574	10-20		4.0
G575	20-30 (partial)		0.0
G576	43-45		0.0
G577	45-80	Slope mantle derived from member, composited	1.0
		Composite of 30 feet of beds, 3.6 miles north of Junction of Highways 18 and 281 on Highway 281	0.5

of selenium, with one exception, in composited samples. Selenium was not detected in two samples, but one contained 4.0 p.p.m. Although judgment as to the seleniferous character of the Elk Butte member must await conclusive data, the available evidence suggests that the selenium content of the member is low.

SELENIUM IN VEGETATION FROM THE VARIOUS FORMATIONS.—During the summer of 1938 vegetation was collected from the Cretaceous formations in South Dakota and analyzed for selenium (tables 14-20).

Several of the samples were taken on the same locations as the geological sections (see map 1). All the samples were taken from formations identified by macro- and microfossils whenever there was a question as to their identity.

Under natural conditions several factors affect the selenium content of vegetation. Beath et al. (1937a, 1937b) have studied the effect of (1) geological formation, (2) age of plants, (3) drying of plants for analysis, (4) stage of growth, (5) part of plants analyzed, and (6) thriftiness of plants, upon the selenium content of the plants determined by analysis. They found that Niobrara shale produced much more highly seleniferous *Astragalus racemosus* (Tium racemosum of Rydberg) than did Benton shale and that woody aster (*Xylorhiza Parryi*) grown on Pierre shale contained much more selenium than did that grown on Fort Union shale. Older plants of *Astragalus bisulcatus* (*Diholcos bisulcatus* of Rydberg) were found to contain more selenium than young ones, age being determined by size and physical character of the roots. A loss of selenium upon drying of certain highly seleniferous plants was found, and Beath (personal communication) stated that there is no definite relationship between the original selenium content of a plant and the loss of selenium on drying. On the other hand, crops such as cereals, grasses, and vegetables were reported not to lose selenium upon drying. A considerable variation in selenium content at different stages of growth of *Xylorhiza Parryi*, *Astragalus pectinatus*, *Oenopsis condensata*, and *Aster comutatus* was reported. (Work at this laboratory—unpublished data—has shown that variation occurs also in *Aster multiflorus*, *Grindelia squarrosa*, and in several less seleniferous plants.) A study of *Astragalus pectinatus* showed that the seeds contained a greater amount of selenium than the roots, which in turn contained more of the element than the leaves and stems, which were about equally seleniferous. A regrowth of *Astragalus racemosus* was found to be much more highly seleniferous than the roots or dry stems. A favorable growing season was found to be conducive to higher selenium uptake than an unfavorable season.

Beath et al. (1935) found that certain plants, such as *Astragalus racemosus*, *Astragalus bisulcatus*, and some species of *Oenopsis*, *Xylorhiza*, *Mentzelia*, and *Stanleya*, were capable of taking large amounts of selenium from the rock or soil in which they grew, whereas other plants, such as the grains and grasses, took up only very small amounts. He found also that there was no correlation between the selenium content of the less seleniferous plants and the total selenium in the soils upon which they grew. He concluded, therefore, that the element occurred naturally in a very insoluble form which most plants could not use. However, some plants, like the *Astragali* and others mentioned above, are capable of absorbing (in an as yet unknown manner) this insoluble form, converting it into organic selenium, in which form it may be deposited in the soil when the plant dies. As this

organic form, it could then become available to other plants. Olson and Moxon (unpublished data) have found that the selenium content of the more common grain plants is very closely associated with the organic selenium content of the soil in which they grow, a fact which is in agreement with Beath's statements concerning the form of selenium as related to its absorption by plants.

Examination of the geological data presented in this paper shows considerable variation within formations as well as between formations. This indicates that a considerable variation in the selenium content of plants growing on different levels of the formation may be expected.

known to contain considerable selenium. Although *Mentzelia decapetala* does not usually absorb much of the element, it is considered important in indicating the presence of selenium in soil and rock. These six plants, therefore, compose the greater part of the collection.

Until they are considerably weathered, certain formations support little if any vegetation. Whether this is due to physical or chemical factors, or both, has not been established. The Sharon Springs, Gregory, Agency, and Oacoma members of the Pierre formation are examples of this, and but a few samples were collected on them. Most plants collected on Sharon Springs were found on the upper part of the

TABLE 14. *Vegetation on the Benton Group.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
CARLILE				
V4	7/6/38	<i>Astragalus racemosus</i> Pursh	Early maturity	7.2
GREENHORN				
V65	7/9/38	<i>Astragalus racemosus</i> Pursh	Preblossom	13.8
V244	7/22/38	" " "	Pods shed	60.0
V259	7/22/38	" " "	Pods shed	125.0
V260	7/22/38	<i>Stanleya bipinnata</i>	Early maturity	60.0
V265	7/22/38	<i>Mentzelia decapetala</i> Pursh (<i>Nuttallii decapetala</i> (Pursh) Greene)	Bud	0.0
V267	7/22/38	" " "	Bud	1.0
V66	7/9/38	" " "	Pre-bud	1.6
V266	7/22/38	" " "	Bud	8.0
V257	7/22/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Bud	1.4
V263	7/22/38	" " "	Bud	2.0
V262	7/22/38	" " "	Bud	3.4
V258	7/22/38	" " "	Bud	4.0

These several factors are pointed out because it is essential that they be considered in the examination of the data presented herein.

All plant collections listed here were made in the summer of 1938 from bed rock which was relatively little weathered. The plants were cut off just above the surface of the soil or rock in which they grew, unless otherwise specified. The date of collection, the stage of growth, and part of formation on which they were collected were recorded. The plants were dried at 50°-60°C. for 24-36 hours. They were then ground in a Wiley mill, and a representative portion was analyzed for selenium by the method described by Moxon (1937). Since no attempt will be made here to correlate the selenium content of the plants with the various stratigraphic positions within the formations, data pertaining to this phase of the work have been omitted from this paper.

Of those plants which are capable of taking up large quantities of selenium, *Astragalus racemosus*, *Aster multiflorus*, *Gutierrezia sarothrae*, and *Stanleya bipinnata* are the most important in South Dakota. *Grindelia squarrosa* also has in some instances been

member where the conditions are most favorable for vegetative growth.

The fact that *Astragalus racemosus*, *Stanleya bipinnata*, and *Mentzelia decapetala* grow practically without exception only on seleniferous zones, and that *Aster multiflorus* requires a fairly large amount of moisture for vigorous growth, added to the difficulty in obtaining samples in many cases.

Byers et al. (1938) reports *Stanleya pinnata* with 260 p.p.m. of selenium growing on the Greenhorn formation in Pueblo county, Colorado. Although considerable selenium was found to occur in the Graneros and Carlile formations, no toxic vegetation was reported. A study of table 14 shows that *Astragalus racemosus* and *Stanleya bipinnata* may in some instances exhibit a fairly high selenium content when growing on the Greenhorn in South Dakota. The fact that such cases are rarely found, and that when they are found the plants are restricted to a very narrow zone in the formation, leads the authors to believe that the Greenhorn formation offers no problem in South Dakota. The Graneros and Carlile apparently do not produce much seleniferous vegetation.

TABLE 15. *Vegetation on the Niobrara Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V6	7/6/38	<i>Astragalus racemosus</i> Pursh	Blossom	24.0
V213	7/20/38	" " "	Varied	50.0
V12	7/6/38	" " "	Pods maturing	80.0
V102	7/12/38	" " "	Pods mature	100.0
V142	7/15/38	" " "	Pods maturing	150.0
V30	7/6/38	" " "	Pods maturing	180.0
V35	7/6/38	" " "	Pods maturing	186.0
V136	7/13/38	" " "	Pods maturing	315.0
V32	7/6/38	" " "	Pods maturing	320.0
V41	7/6/38	" " "	Pods maturing	500.0
V13	7/6/38	" " "	Pods maturing	526.0
V132	7/13/38	" " "	Pods maturing	550.0
V101	7/12/38	" " "	Pods mature	550.0
V112	7/12/38	" " "	Pods mature	600.0
V228	7/20/38	" " "	b	625.0
V172	7/16/38	" " "	b	760.0
V189	7/18/38	" " "	b	760.0
V177	7/16/38	" " "	b	880.0
V179	7/16/38	" " "	b	1160.0
V175	7/16/38	" " "	b	1960.0
V181	7/16/38	" " "	b	2640.0
V28	7/6/38	" " "	Pods mature	2700.0
V169	7/16/38	" " "	b	2880.0
V166	7/16/38	" " "	b	4100.0
V7	7/6/38	<i>Astragalus bisulcatus</i> A. Gray	Late blossom	2.8
V69	7/9/38	<i>Stanleya bipinnata</i>	Dry	2.0
V113	7/12/38	" " "	Mature	40.0
V42	7/6/38	" " "	b	136.0
V40	7/6/38	" " "	Early maturity	150.0
V37	7/6/38	" " "	Early maturity	200.0
V38	7/6/38	" " "	Early maturity	240.0
V31	7/6/38	" " "	Early maturity	350.0
V39	7/6/38	" " "	Early maturity	376.0
V230	7/20/38	" " "	Dry	385.0
V33	7/6/38	" " "	Early maturity	476.0
V29	7/6/38	" " "	Early maturity	520.0
V148	7/15/38	" " "	Early maturity	525.0
V34	7/6/38	" " "	Early maturity	550.0
V135	7/13/38	" " "	Early maturity	575.0
V36	7/6/38	" " "	Early maturity	626.0
V133	7/13/38	" " "	Early maturity	860.0
V183A	7/18/38	" " "	Mature	2380.0
V8	7/6/38	<i>Aster multiflorus</i> Ait.	Past blossom	7.2
V149	7/15/38	" " "	8-12 inches	19.2
V173	7/16/38	" " "	b	48.0
V183	7/16/38	" " "	b	52.0
V167	7/16/38	" " "	b	90.0
V170	7/16/38	" " "	b	165.0
V184	7/18/38	" " "	Stunted	320.0
V68	7/9/38	" " "	b	366.0
V147	7/15/38	<i>Mentzelia decapetala</i> Pursh (<i>Nuttallii decapetala</i> (Pursh) Greene)	Bud	1.2
V214	7/20/38	" " "	Bud	2.0
V216	7/20/38	" " "	Bud	3.2
V134	7/13/38	" " "	Bud	4.0

TABLE 15.—Cont'd.

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V176	7/16/38	<i>Gutierrezia sarothrae</i> (Pursh) Britt. and Rusby (<i>G. euthamiae</i> T. and G.)	b Preblossom	8.0
V174	7/16/38	" "	Preblossom	8.8
V168	7/16/38	" "	Preblossom	14.0
V178	7/16/38	" "	Preblossom	18.0
V171	7/16/38	" "	Preblossom	22.0
V180	7/16/38	" "	Preblossom	44.0
C182	7/16/38	" "	Preblossom	46.4
V188	7/18/38	" "	Preblossom	220.0
V215	7/20/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Bud	2.0
V229	7/20/38	" " "	Varied	7.0
V236	7/21/38	" " "	Bud	12.0
V186	7/18/38	" " "	Pre-bud (10-14 in.)	260.0
V114 ^a	7/12/38	<i>Juniperus virginiana</i> L.	b	5.4 and 7.0
V185	7/18/38	<i>Artemisia canadensis</i> Michx.	b	6.8
V239	7/21/38	<i>Agropyron smithii</i> Rydb. (<i>A. occidentale</i> Scribn.)	Heads mature	3.0
V187	7/18/38	" "	Mature	27.0
V228	7/21/38	<i>Distichlis stricta</i> (Torr.) Rydb. (<i>D. Spicata</i> Coult. and Nels.)	Heads mature	11.2
V489 ^a	8/8/38	<i>Ulmus americana</i> L.	b	1.0
V490 ^a	8/8/38	<i>Frazinus lanceolata</i> Berek.	b	3.0

^a Analysis of leaves only.^b Stage not recorded.TABLE 16. *Vegetation on Sharon Springs member of the Pierre Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V125	7/13/38	<i>Astragalus racemosus</i> Pursh	Pods maturing	10.0
V97	7/11/38	" " "	Pods mature	48.0
V94	7/11/38	" " "	Pods mature	490.0
V156	7/15/38	<i>Aster multiflorus</i> Ait.	(10-14 in.)	0.0
V157	7/15/38	" " "	(10-14 in.)	1.0
V95	7/11/38	" " "	(10-16 in.)	3.6
V57	7/8/38	" " "	(6-10 in.)	11.0
V93	7/11/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Bud	7.4
V92 ^a	7/11/38	<i>Prunus melanocarpa</i> (A. Nels) Rydb.	b	1.0
V98	7/11/38	<i>Agropyron smithii</i> Rydb. (<i>A. occidentale</i> Scribn.)	Dry	15.0
C14	6/1/38	<i>Astragalus crassicaulis</i> Nutt.	Pods forming	2.0
C16	6/1/38	<i>Aster commutatus</i> T. and G.	(8-12 in.)	4.6

^a Analysis of leaves only.^b Stage not recorded.

An examination of table 15 shows that in every case vegetation collected on the Niobrara contained selenium. *Astragalus racemosus* and *Stanleya bipinnata* exhibit high selenium contents almost without exception, the average content of the samples collected being 941.5 p.p.m. and 493.6, respectively. For *Aster multiflorus*, *Grindelia squarrosa*, and *Gutierrezia sarothrae* the average selenium contents are 133.4 p.p.m., 70.2 p.p.m., and 47.6 p.p.m., respectively. Grasses and trees also show the presence of the element in

comparatively high amounts. There is a considerable area of Niobrara outcrops and soils derived from them in South Dakota, especially around the Black Hills. Most of the area supports a fairly substantial growth of vegetation, a large part of which consists of seleniferous plants. Many cases of selenium poisoning have been reported on this formation.

As has already been stated, the occurrence of any vegetation on Lower Sharon Springs outcrops is almost rare. On the upper part of the member plants are

TABLE 17. *Vegetation on Sully member of the Pierre Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
AGENCY				
V487	8/7/38	<i>Aster multiflorus</i> Ait.	a	4.0
OACOMA				
C17	6/1/38	<i>Astragalus racemosus</i> Pursh.	Blossom	860.0
V292	7/25/38	" " "	Pods shed	20.0
V486	8/7/38	<i>Gutierrezia sarothrae</i> (Pursh) Britt. and Rusby (<i>G. euthamiae</i> T. and G.)	Pre-blossom	1.0
C19	6/1/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Pre-blossom	5.0
C13	6/1/38	<i>Amorpha</i> sp. ?	Pre-blossom	1.4
VERENDRYE				
V232	7/20/38	<i>Astragalus racemosus</i> Pursh.	Pods mature	39.0
V207	7/19/38	" " "	Pods mature	44.0
V483	8/7/38	<i>Aster multiflorus</i> Ait.	a	1.0
V484	8/7/38	" " "	a	10.0
V488	8/8/38	" " "	Bud	10.0
V485	8/7/38	<i>Gutierrezia sarothrae</i> (Pursh) Britt. and Rusby (<i>G. euthamiae</i> T. and G.)	Pre-blossom	0.0

TABLE 18. *Vegetation on Virgin Creek member of the Pierre Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V59	7/8/38	<i>Astragalus racemosus</i> Pursh.	Pods maturing	13.0
V361	8/1/38	" " "	Pods shed — partially dry	24.0
V258	8/1/38	" " "	Pods shedding	44.0
V375	8/3/38	" " "	Pods mature	500.0
V362	8/1/38	<i>Aster multiflorus</i> Ait.	Bud	5.0
V359	8/1/38	" " "	Early blossom	6.0
V376	8/3/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Blossom	10.0
V360	8/1/38	<i>Stanleya bipinnata</i>	Pods mature	27.0
V377	8/3/38	<i>Xanthium pennsylvanicum</i> Waller	Early maturity	4.0
V378	8/3/38	<i>Agropyron smithii</i> Rydb. (<i>A. occidentale</i> Scribn.)	Heads mature	0.0
V379	8/3/38	<i>Salsola pestifer</i> A. Nels.	Green	4.0
V380	8/3/38	<i>Helianthus</i> sp.	Seeds mature	0.0
V381	8/3/38	<i>Lygodesmia juncea</i> (Pursh). D. Don.	Past blossom	1.0
V382	8/3/38	<i>Lepaulenia marginata</i> (Pursh). Niewl. (<i>Euphorbia marginata</i> Pursh)	a	3.0
V383	8/3/38	<i>Melilotus officinalis</i> (L.) Lam	Late blossom	4.2
V384	8/3/38	<i>Brameria angustifolia</i> (D.C.) Heller	Seeds mature	1.0
V385	8/3/38	<i>Solidago pallida</i> (Porter.) Rydb.	a	4.0
V386	8/3/38	<i>Astragalus mollissimus</i> Torr.	Pods mature	1.0
V387	8/3/38	<i>Amaranthus retroflexus</i> L.	Early maturity	0.0

a Stage not recorded.

TABLE 19. *Vegetation on Mobridge member of the Pierre Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V347	7/28/38	<i>Astragalus racemosus</i> Pursh.	Pods shed	0.0
V349	7/28/38	" " "	Pods shed	0.0
V462	8/6/38	" " "	Pods shed	0.0
V477	8/6/38	" " "	Pods shed	0.0
V56	7/8/38	" " "	Early pre-blossom	0.8
V355	7/30/38	" " "	Pods shed	1.5 and 4.0
V478	8/6/38	" " "	Pods shed	3.0
V474	8/6/38	" " "	Pods shed	4.0
V476	8/6/38	" " "	Pods shed	4.4
V526	8/9/38	" " "	b	8.0
V524	8/9/38	" " "	b	10.0
V391	8/3/38	" " "	Pods shed	10.0
V339	7/27/38	" " "	Pods shed	10.0
V323	7/26/38	" " "	Pods mature	16.0
V43	7/7/38	" " "	Pre-blossom	17.0
V482	8/6/38	" " "	b	26.0
V342	7/27/38	" " "	Pods shed	32.0
V294	7/26/38	" " "	Pods mature	48.0
V340	7/27/38	" " "	Pods shed	48.0
V338	7/27/38	" " "	Pods shed	64.0
V341	7/27/38	" " "	Stunted	75.0
V491	8/8/38	" " "	Pods shed	125.0
V481	8/6/38	" " "	Pods shed	224.0
V403	8/3/38	" " "	Pods shed	250.0
V351	7/29/38	" " "	Pods shed	250.0
V100	7/12/38	" " "	Pods mature	325.0
V343	7/27/38	" " "	Stunted	641.0
V529	8/10/38	" " "	Pods shed	1100.0
V414	8/4/38	<i>Astragalus</i> sp ?	b	0.0
V457	8/5/38	<i>Aster multiflorus</i> Ait.	b	0.0
V49	7/7/38	" " "	(12-16 in.)	0.8
V356	8/1/38	" " "	Bud	1.0
V547	8/10/38	" " "	Bud	3.2
V492	8/8/38	" " "	Bud	4.0
V292	8/3/38	" " "	b	6.0
V555	8/10/38	" " "	Bud	9.2
V45	7/7/38	<i>Mentzelia decapetala</i> Pursh. (<i>Nuttallii decapetala</i> (Pursh) Greene)	Varied	0.6
V317	7/26/38	" " "	Bud	1.0
V283	7/23/38	" " "	Bud	1.0
V55	7/7/38	" " "	Pre-bud	1.6
V548	8/10/38	" " "	Bud	6.0
V393	8/3/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Bud	0.0
V282	7/23/38	" " "	Bud	8.0
V479 ^a	8/6/38	<i>Juniperus virginiana</i> L.	b	0.0
V512	8/8/38	<i>Bouteloua curtipendula</i> (Michx.) Torr. (<i>Atheropogon curtipendulum</i> Fourn.)	Heads mature	0.0
V538	8/10/38	<i>Chenopodium album</i> L.	Early maturity	1.0
V539	8/10/38	<i>Panicum capillare</i> L.	Early maturity	1.4
V551	8/10/38	<i>Agropyron smithii</i> Rydb. (<i>A. occidentale</i> Scribn.)	Late maturity	10.0

^a Analysis of leaves only.^b Stage not recorded.

fairly abundant. Table 16 shows that in some cases *Astragalus racemosus* of relatively high selenium content may be found. Since the upper Sharon Springs has been found to be highly seleniferous, and since it produces considerable vegetation, some of which is highly seleniferous, this part of the member is considered a possible source of selenium poisoning. The lower part of the member, although it is also highly seleniferous, probably presents no problem, because of its lack of vegetation.

Table 17 gives the selenium content of plants found on the Sully member of the Pierre. Although certain zones of the Sully member are seleniferous, they are

The relatively small area of Elk Butte exposed in the area studied and the low selenium content of the member where it was examined account for the small number of plants collected on it. (See table 20.) This member of the Pierre is not considered dangerous in selenium poisoning.

SUMMARY

Selenium occurs in all Cretaceous formations in South Dakota from the Dakota sandstone up through the Pierre formation.

The selenium content of the Dakota, Graneros, Greenhorn, and Carlile formations is low.

TABLE 20. *Vegetation on Elk Butte member of the Pierre Formation.*

No.	Date	Vegetation	Stage	Se content, oven-dried basis
V580	8/12/38	<i>Aster multiflorus</i> Ait.	Bud	0.0
V579	8/12/38	<i>Grindelia squarrosa</i> (Pursh) Dunal.	Bud	0.0
V578	8/12/38	<i>Agropyron smithii</i> Rydb. (<i>A. occidentale</i> Scribn.)	Mature	0.0

not as a rule highly so. Furthermore, on the Gregory marl and on the Oacoma zone vegetation is sparse. The generally low selenium content of the Sully member along with the sparseness of vegetation leads the authors to believe that danger from selenium poisoning of this member is slight.

Aside from the chalky zone found occurring in the upper Virgin Creek member of the Pierre, most of the member is not capable of producing highly seleniferous vegetation. However, the chalky zone is somewhat seleniferous, and it is believed to be responsible for some cases of selenium poisoning. Samples V360 and V375 (table 18) were found growing on this chalky bed.

In table 19 are listed the plants collected on the Mobridge member of the Pierre together with their selenium contents. Examination of the data concerning *Astragalus racemosus* shows considerable variation. Several specimens of *Astragalus racemosus* are high in selenium, but several others are very low in selenium. This corresponds well with the fact that the Mobridge member varies in selenium content at different locations. Where the member is highly seleniferous, considerable vegetation is found and selenium poisoning is common. Considerable grain high enough in selenium to be toxic is grown on the soils derived from this formation.

The selenium content of the Fort Hays member of the Niobrara formation is low, whereas that of the Smoky Hill member is high.

The selenium content varies greatly in the different members of the Pierre formation; it is high in the Sharon Springs, locally high in the Mobridge, and generally low in the Sully and Virgin Creek.

Geographic variation in selenium content of the Mobridge member of the Pierre suggests correlation between thickness of the member and selenium content—high concentrations occurring where the member is thin and low concentrations where it is thick.

Factors determining the selenium content of vegetation are discussed, and a list of the more important seleniferous plants in South Dakota is given.

Analyses of vegetation collected on the various geological formations are given. A close relationship between the formation, type of vegetation, and selenium content of vegetation is found to exist.

Seleniferous formations which themselves are capable of producing vegetation or which break down readily to soils which will support vegetation are found to be important in selenium poisoning of livestock.

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GENERAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE GENERAL SECTION
OF THE BOTANICAL SOCIETY OF AMERICA, RICHMOND, VIRGINIA,

DECEMBER 28 TO 30, 1938

HETEROTHALLISM IN ASCOBOLUS GEOPHILUS SEAYER.
Edwin M. Betts and Samuel L. Meyer, University of Virginia, Charlottesville, Va.—*Ascobolus geophilus* Seaver is found to be heterothallic. Ascocarps do not develop from monospore cultures but may be produced by crossing mycelia from spores of opposite strains. The mycelia are produced by germinating single spores on soil decoction agar. Crossing of mycelia from spores of opposite strains in single asci shows that four spores in an ascus are of plus (minus) strain and four spores are of minus (plus) strain. The results indicate that sex factors may be segregated at either the first or second divisions.

THE ARRANGEMENT OF SPORES FOR SEXUAL STRAINS IN TWENTY-SEVEN ASCI OF ASCOBOLUS CARBONARIUS KARST. *Edwin M. Betts and Samuel L. Meyer, University of Virginia, Charlottesville, Va.*—*Ascobolus carbonarius* Karst. has been found to be heterothallic. Sex segregation always occurs at the first nuclear division. Spores from twenty-seven asci were segregated in the order of their occurrence in the ascus and numbered 1, 2, 3, 4, 5, 6, 7, 8, from base to apex. The mycelia developed from single spores from one ascus were grown in all possible combinations with single spore cultures from the other asci. Twenty of the asci showed spores 1, 2, 3, 4 to be of plus (minus) strain, while seven of the asci showed spores 1, 2, 3, 4 to be of minus (plus) strain. Spores 5, 6, 7, 8 of the twenty asci were of minus (plus) strain, while spores 5, 6, 7, 8 of the seven asci were of plus (minus) strain. These results indicate that sex segregation in *Ascobolus carbonarius* Karst. is not a chance distribution.

SOME INTERESTING CYTOLOGICAL ASPECTS OF CONJUGATION AND GERMINATION. *W. R. Hatch, Dartmouth College, Hanover, N. H.*—Conjugation in plants, a phenomenon of the very greatest import, has hardly received the attention it merits. This paper is an attempt to rectify this condition in so far as any cytological examination can. The material, excellent for the purpose, is a water mold *Allomyces arbuscua*. The initial collapse of the membranes between any two conjugants occurs in a limited and sharply circumscribed region at the base of the cilia. Then the two gametes flow together through an ever widening canal. The nuclei by reason of their rhizoplastic connection, swing together. Their fusion, however, is not immediate and must wait upon the dissolution of the nuclear cap, a bulky cytoplasmic structure.

The germination of a uni-cell, be it zygote or zoospore, likewise merits study. Here it is apparent that the external manifestations of germination (the extension of the rhizoid and germ tube) are anticipated

and conditioned by internal change in which the dissolution of the nuclear cap plays a prominent role.

SO-CALLED SEX CHROMOSOMES IN *LYCHNIS ALBUM*. *Henry Wilhelm Jensen, Ashville Farm School, Swananoa, N. C.*—A reinvestigation of *Lychnis* (*Melandrium*) *album* revealed considerable evidence which makes the existence of sex chromosomes in this species very doubtful. The X-Y chromosome complex was carefully studied with improved techniques and was found to be basically a tetrapartite association, made up of two pairs of chromosomes whose normal method of division had been suppressed. Previous hybridization of the species seems to have initiated these meiotic peculiarities which have been mistaken by some investigators for sex chromosomes. Attention is drawn to the similar conditions found in the dioecious species of *Rumex* and *Lychnis*. Comparison is also made between the quite normal meiotic behavior of monotypic unisexual species and the distinctly peculiar meiotic conditions found in the dioecious species of large and variable genera—particularly such genera in which both perfect and unisexual forms abound. From his numerous investigations the author presents arguments for his opinion that sex chromosomes, as currently conceived, are not found in the higher plants.

CHROMOSOME STUDIES IN THE GENUS *CALOCHORTUS*. *John M. Beal, University of Chicago, Chicago, Ill.*—A report of the somatic chromosomes, and in large part of the meiotic chromosomes at first metaphase, in 26 species and 12 varieties of *Calochortus*. The diploid numbers observed in the various species are 12, 14, 16, 18, 20, 28, 32, and 40. Although size and form variations of the chromosomes are fairly marked between some of the species, these are less conspicuous than the variation in numbers. The relation of karyotypes to taxonomic arrangement will be discussed.

THE CHROMOSOME NUMBER OF SOME TULIPA HYBRIDS. *R. Bamford, G. B. Reynard, and J. M. Belows, University of Maryland, College Park, Md.*—Most species and varieties of *Tulipa* have diploid chromosome numbers, but there are a few polyploid forms including some triploids and one pentaploid. The triploids are all self-sterile, but cross-fertile with some of the diploids. The results of such reciprocal crosses between diploids and triploids indicate that the male and female gametes of the triploid are of different types. Although the evidence is meager, a similar situation apparently exists in the pentaploid.

CHROMOSOME NUMBER AND HYBRIDIZATION IN *GLADIOLUS*. *Ronald Bamford, University of Mary-*

land, College Park, Maryland.—The species and varieties of *Gladiolus* have previously been shown to be in a polyploid series (n=15), and they range from diploid to eneaploid types. Attempts have been made to hybridize those having the same chromosome number and also those having different chromosome numbers. The results indicate that the more nearly two types are related, on the basis of chromosome number, the higher the number of progeny. The exceptions to this generalization are the triploid and the pentaploid forms.

CHROMOSOMES OF CRASSULACEAE. J. T. Baldwin, Jr., College of William and Mary, Williamsburg, Va.—The Crassulaceae, highly variable in their chromosomes, have a known 2n-range of 8 to ca. 500. Some of the species have more than a single chromosome number. For example, the following somatic counts have been made: in *Sedum ternatum*, 16 and 32; in *S. Nevii*, 12 and 28; in *S. anglicum*, 48 and 144; in *S. dasyphyllum*, 28, 42, and 56; in *S. hispanicum*, 14, 28, 30, and 40. Recognition of trends in the chromosomal evolution of the Crassulaceae gives an additional basis for interpreting certain taxonomic categories in the family.

OENOTHERA MUT. CONTRACTA, A NEW GENE MUTATION IN THE DECIPiens GENOME OF OENOTHERA MUT. ERYTHRINA. George H. Shull, Princeton University, Princeton, N. J.—*Oenothera* mut. *erythrina* is one of several mutational derivatives of *Oe. Lamareckiana* which differ genetically from *Lamareckiana* by the loss of one of the *Lamareckiana* lethals. These forms give progenies consisting of the parent type (*erythrina* in this instance) and a homozygous form (*decipiens*) in a ratio approximating 2:1. Cytologically these two phenotypes have the typical number of chromosomes (14), but differ in that *erythrina* has a circle of six (or sometimes eight) chromosomes and four (or three) pairs, while *decipiens* has seven pairs.

In the spring of 1938 one of many progenies from *erythrina* parents which have come by repeated selfings of *erythrina* ancestors was observed to consist of 61 *erythrina* and 40 mut. *contracta*, no *decipiens* plants being included. It is obvious that a gene mutation has taken place which was carried as a recessive in the *erythrina* parent of this progeny and which became manifest in the *decipiens* component of the offspring because of the location of this new gene in a chromosome characteristic of the *decipiens* genome. On this assumption all the *erythrina* plants of this family (No. 36428) should yield, on selfing, again a ratio of 2 *erythrina* to 1 *contracta*. Mut. *contracta* is an extremely crinkled form which can be recognized in very early rosette stages, but none has yet bloomed.

CYTOLOGICAL EFFECTS OF COLCHICINE UPON DIVISION OF THE GENERATIVE CELL IN POLLEN TUBES OF LILIUM. O. J. Eigsti, University of Oklahoma. Norman, Oklahoma.—The division of generative cells in pollen tubes of *Lilium* varies somewhat from the division of cells in sporophytic tissue. A generative nucleus is surrounded by a cytoplasmic sheath which is not limited

by a cell wall. There is some difference of opinion regarding the mechanics of division of the generative cell. In this study, neither a definite spindle fiber nor definite cell plate formation was clearly differentiated, but the chromosomes apparently were arranged on a "plate" similar to a regular mitosis. Since it has been shown that colchicine affects spindle fiber formation and hence the mitotic process, it is thought that the application of colchicine to pollen tubes of *Lilium* might yield new information concerning the problem of division of the generative cell as well as mitotic variations of the microgametophyte. Division of the generative cell in pollen tubes of *Lilium* when treated with solutions of colchicine showed the following effects: (1) the chromosomes were not arranged on a "plate" but occurred at random, (2) the cytoplasmic sheath was spherical and rounded instead of elliptical and elongate, (3) segregation of chromosomes was arrested, and (4) colchicine affected the cytoplasmic portion of the generative cell and inhibited nuclear division, which brought about chromosome variations.

CYTOPLASMIC INCLUSIONS IN THE MALE GAMETES OF LILIUM. Lewis E. Anderson, Duke University, Durham, N. C.—The development of the male gametes of three species of *Lilium* were studied in pollen tubes germinated both artificially and in the styles. In all three species the male gametes are complete cells, consisting of a nucleus surrounded by a layer of cytoplasm which is delimited by a definite membrane. Both plastids and mitochondria are present in the cytoplasm of each gamete. The inclusions are derived from the generative cell.

NUCLEAR SIZE IN THE DIFFERENTIATING SPIRAL ELEMENTS OF RICINUS COMMUNIS. Flora Murray Scott, University of California at Los Angeles.—The volume of meristem nuclei in the shoot apex of *Ricinus communis*, calculated from diameter measurement, is 38 cubic microns. The size of the nuclei in differentiated parenchyma tissue (e.g. pith) averages about eight times this number (332 cubic microns). In the largest differentiating spiral vessels the nuclei reach the relatively enormous volume of 10,000, 18,000, 33,000, and in one case, 55,000 cubic microns. In various stages of mitotic division it is seen that the number of chromosomes remains constant. The increase in size is therefore due to additional nuclear sap. The volume of the nucleolus does not increase proportionately.

INFLUENCE OF AUXONES ON HYPOCOTYLEDONARY BUD INITIATION IN FLAX. George K. K. Link and Virginia Eggers, University of Chicago, Chicago, Ill.—The decapitated hypocotyl of flax produces buds each of which is initiated by division of one epidermal cell without preceding cell enlargement. First substantial change noted is protoplasmic increase. Rate and frequency of bud initiation are, in part, functions of the metabolic status of the hypocotyl, amounts of light playing a more decisive role than amounts of mineral nitrogen. Ether extracts of epicotyls, and indole-3-acetic acid, applied in lanolin to the decapitated hypocotyl affect both frequency and rate of its bud

initiation. In addition to inhibitory and depressant effects reported by us in *Nature* 142: 398-399, 1938, it was found that certain concentrations of auxins increase both number and rate of bud initiation. Limiting concentrations of these stimulatory effects depend upon the metabolic status of the hypocotyl, amount of light being an important conditioning factor. It is suggested that auxins affect cell division and organ differentiation only indirectly through influence upon production and movements of cell division auxones (cytomerisones) and, possibly, of growing-point differentiating auxones (blastocalines).

THE BIOLOGICAL SPECTRUM OF COLORADO SAND HILLS. Francis Ramaley, University of Colorado, Boulder, Col.—Collections of vascular plants made in the sand hills of northeast-central Colorado now number 208 native species. Arranged by life forms according to Raunkiaer, the biological spectrum is as follows: Succulents, 1; Epiphytes, 0; Phanerophytes, Mega- and Meso-, 0; Phanerophytes, Micro-, 2; Phanerophytes, Nano-, 5; Chamaephytes, 1; Hemicryptophytes, 49; Geophytes, 20; Hydro-Helophytes, 6; Therophytes, 16; Total, 100. Vegetation of the Colorado sand hills is distinctly hemicryptophytic (49 per cent), as would be expected from the latitude, while the dry and cold winters favor geophytes (20 per cent). The biological spectrum resembles closely that of Denmark, but it is similar also to spectra of various sandy-soil stations in foothill districts of Colorado.

SUBCLIMAX PRAIRIE. W. E. Loomis and A. L. McComb, Iowa State College, Ames, Iowa.—All sections of Iowa contain unprotected native forests which have largely escaped injury during the recent dry cycle. Forests have been observed to arise spontaneously on Iowa prairie sites when the grass is destroyed and fires controlled. Under present climatic conditions the prairies of Iowa and eastward must, therefore, be considered to be subclimax formations. On typical prairie soils of the Marshall, Webster, and Clyde types, current or recent invasion of grasslands by trees is strikingly correlated with erosion. Large scale pot experiments indicate that soil fertility, particularly soil nitrogen, is responsible for this correlation. Added nitrogen on eroded soils has made little difference in the first year development of seedlings of *Quercus borealis*, *Fraxinus lanceolata*, *Pinus Banksiana*, and *Juglans nigra*, but has meant the difference between good growth and near failure of *Avena sativa*, used as an index of grass response. Our experiments and observations indicate that the decisive factor in the persistence of subclimax prairie is the high original and maintained fertility of uneroded glacial drift soils, which stimulates such a vigorous growth of grasses and herbs as to effectively check the spread of forest species. Erosion, by sharply diminishing the nitrogen content of the soil, has reduced the growth of grasses and permitted forests to become established. Once established, the forest has maintained itself, even on the most exposed sites of central and west-central Iowa.

A STUDY OF A TUPELO GUM SWAMP. William T. Penfound, Tulane University, New Orleans, La.—A pure stand of tupelo gum, near Huntsville, Alabama, was studied in July, 1938. This swamp forest has a dense canopy which envelopes the nearly barren forest floor in almost continuous shade. The slender trees are 104 feet in height and nearly 200 years old. The average diameter of the swollen base is 16.5 inches, but at the height of one's head, the trunk narrows abruptly to 9.0 inches. The north faces of the trees are clothed with several communities of liverworts and mosses, but these are absent on the south exposure, despite the hydric conditions obtaining in the swamp. Only twelve arborescent species and seven herbaceous species were found in the swamp. The paucity of herbaceous species is due probably to the unfavorable growing conditions occasioned by the dense shade and the long hydropereod existing in this swamp.

RAINFALL CONDUCTION BY TRUNKS OF TREES IN A TROPICAL RAIN FOREST. Paul D. Voth, University of Chicago, Chicago, Ill.—Tin cups were soldered to fit around the trunks of shrubs and small trees growing in a clearing or in the tropical rain forest on Barro Colorado Island, Gatun Lake, Canal Zone. Water collected by the cups was conducted to suitable containers by tubes. The volume of rain water collected was measured every twelve hours for a period of two weeks. From these records the amount of rainfall was calculated. The control cup collected approximately as much water as the standard rain gauge nearby. A cup attached to a small tree growing at the edge of the forest collected as much as five times the amount recorded by the rain gauge or the amount collected by the control cup. A comparable amount of water was collected by a cup attached to a young tree growing under the canopy of the tropical rain forest. Cups were also placed in tandem on the trunk of a shrub in the clearing.

GROWTH RINGS IN HEMLOCKS. G. S. Avery, Jr., H. B. Creighton, and C. W. Hock, Connecticut College, New London, Conn.—Work is in progress on attempted cross-dating, etc., of growth rings in hemlock trees destroyed in the Connecticut Arboretum at Connecticut College during the hurricane of September 21. Since some of the 200-year-old trees grew on dry rock ledges and others in wet places, an opportunity is afforded to compare the effects which differences in habitat have on annual rings in trees growing in the same locality.

BRYOPHYTIC SUCCESSION ON ROCKS IN THE REGION OF MOUNTAIN LAKE, VIRGINIA. Paul M. Patterson, Hollins College, Va.—A total of 371 errant blocks of Clinch sandstone were studied in their entirety in random groups at eleven stations above 3000 feet elevation for the occurrence, relative abundance, and examples of active succession of bryophytes. General observations were made on a much larger number. Cliffs, fissured rocks, and boulders mechanically collecting humus were excluded. Although more than 50 bryophytes were found on this restricted substrate,

only six mosses play the major successional role, the order being established by several lines of evidence. Successions depend in large part upon the degree of mesic conditions prevailing at the habitat or micro-habitat, the pioneers being strongly xeric, the later successions progressively less xeric. Although a number of interesting variations occur, involving other species of bryophytes, the typical series commencing with the climax lichen stage is as follows: (1) *Umbilicaria*, *Gyrophora*, *Parmelia*; (2) pioneer mosses: *Uloa Americana*, *Hedwigia albicans* and/or *Dicranum fulvum*; (3) intermediate: *Dicranum scoparium*; (4) climax: *Thuidium delicatulum* and/or *Hylocomium brevirostre*; (5) vascular plants. More than 30 vascular plants were noted as pioneers, the most frequent being *Polypodium vulgare*.

PROGRESS IN THE COLOR PHOTOGRAPHY OF PLANTS. P. L. Ricker, Bureau of Plant Industry, Washington, D. C.—Photographing in natural colors has progressed rapidly in the past five years so that natural color motion and still pictures of material desired for class use can be made by anyone at a minimum of cost. Recent improvements in projectors give brilliant pictures up to one hundred feet. The latest and least expensive development of two by two inch color slides on 35 mm. color film made with the best type of miniature camera with ground glass focusing enables persons to make their own color slides while on field trips at a cost of not more than twenty cents each. Hair line focusing at distances of from 6½ inches to infinity is possible. Natural size and four times enlargement of small objects can be made in the field, and in the laboratory magnification up to twenty times may be obtained by using extension tubes with the lens. The camera may be attached to a microscope for micro-color work. (Twenty-five or more slides adapted to use in general botany, mycology and ecology will be shown.)

THE ECOLOGICAL IMPORTANCE OF HEMLOCK IN RELATION TO THE CLIMAX FOREST OF SOUTHERN NEW ENGLAND. George E. Nichols, Yale University, New Haven, Conn.—Hemlock planting experiments have been conducted for twelve years in the Yale Natural Preserve, a 150-acre tract of rugged country near New Haven completely wooded with second-growth hardwoods, chiefly oaks. Approximately 25,000 mostly 4-year seedlings planted indiscriminately throughout the area during this period show approximately 98 per cent survival. Growth has been persistent under practically all habitat conditions, although measurements of selected groups of trees show significant variations under different conditions of light and soil moisture. These facts, coupled with the abundant natural reproduction which has taken place during recent years under and around the only two large woodland hemlocks in the vicinity, tend to substantiate the inference that in former days, until wiped out by lumbering and fire, hemlock was a prominent species both in this and in many other areas of "sprout hardwoods" throughout southern New England.

RELATION OF LATITUDE TO THE GROWTH OF TIMOTHY. Morgan W. Evans, Bureau of Plant Industry, U. S. D. A., Wooster, Ohio.—For the purpose of obtaining information in regard to the growing habits of timothy as affected by latitude, plants of 9 selections, ranging by fairly uniform gradations from very early to very late, were grown at each one of 10 stations located at intervals extending from Gainesville, Florida, to Ft. Vermilion, Alberta, Canada.—In the South, selections which are progressively later had progressively shorter stems; in the North, the stems of the later selections grew to as great, or even greater lengths than those of the early selections.—For the earliest selections, the season for blooming progressed northward as the season advanced. For the latest selections, which require a longer day than earlier ones for the development of culms and inflorescences, blooming occurred first at some mid-latitude; from this latitude, the season for blooming progressed both toward the North as the temperatures became suitable for growth, and toward the South as the number of hours of daily illumination gradually increased.—At a southern station, late selections produced smaller yields of hay than early selections, which are better adapted to the relatively short days occurring there. At a northern station, both early and late selections produced relatively large yields.

VASCULAR TISSUES IN THE GAMETOPHYTE OF PSILO-TUM. John E. Holloway, University of Otago, Dunedin, New Zealand. (Introduced by A. J. Eames).—The gametophyte of *Psilotum* is of the same form as, but more stoutly growing than that of *Tmesipteris*. As elongation proceeds there is a slow increase in width of the apex. When the forward end has reached a diameter of at least one millimeter, a definite axial conducting strand is initiated immediately behind the apex. The apex is intermittently active. When comparatively inactive, it ceases to form the strand, and the fungal endophyte then extends forward in advance of the strand. This may happen several times during the growth of the gametophyte. When mature, the strand is of considerable size and usually possesses from one to three, centrally placed, lignified, much elongated, annular tracheids, the strand as a whole being surrounded by a clearly defined endodermis.

GERMINATION AND EARLY STAGES OF THE GAMETOPHYTES OF HYMENOPHYLLUM AND TRICHOMANES. Alma C. Stokey, Mount Holyoke College, South Hadley, Mass.—The primary divisions of the *Hymenophyllum* spore occur usually before discharge from the sporangium. The first wall divides the spore about equally. One cell broadens and is divided by a wall perpendicular to the first. By unequal growth of the three cells a 3-angled prothallium is formed which becomes definitely 3-pointed after the rupture of the spore coat. A secondary wall in each projection forms a tip cell which may develop into rhizoid, filament, or plate. Germination in *Trichomanes nitidum* follows the *Hymenophyllum* type, but two other species showed the "tripolar" type. The spore

becomes 3-pointed after rupture of the spore coat but before septation, with the first walls cutting off the tips of the points. In *T. maximum* one wall was usually suppressed or delayed. The *Trichomanes* type appears to be derived from that of *Hymenophyllum* by the suppression of the primary tri-radiate walls. Examples of the suppression of the primary wall were found in five species of *Hymenophyllum*. The first set of walls in *Trichomanes* corresponds to the second set in *Hymenophyllum* in its relation to the three points and to the valves of the spore coat.

✓ A STUDY OF THE MALE GAMETOPHYTE OF *REGNELIDIUM DIPHYLLUM* LINDMAN. Noe L. Higginbotham, Columbia University, New York, N. Y.—*Regnellidium diphylum*, found only in Brazil, is a monotypic genus of the Marsileaceae. The uninucleate thick-walled microspores, which are nearly spherical with a slightly projecting apex, begin germination about 1½ hours after release from the sporocarp. A unique feature is the formation of a second prothallial cell from a division of the large central cell, since in *Pilularia* and *Marsilea*, the only other genera of the family, second prothallial cells, where found, arise from division of the first prothallial cells. As in the above genera, each of the two antheridia formed within the microspore wall consists of 3 wall cells and 16 spermatocytes, so that the mature male gametophyte in all has 2 prothallial cells, 2 inner and 4 outer wall cells, and 32 spermatocytes in 2 groups. In *Regnellidium* the mature spermatozooids escape through a break in the apex about 14–16 hours after sporocarp dehiscence. The comparatively few coils in the multiciliate spermatozooids show a greater similarity to *Pilularia* than to *Marsilea*.

✓ INFLORESCENCE AND FLORAL ANATOMY AND MORPHOLOGY OF *LEITNERIA FLORIDANA* CHAPM. T. T. Earle, Newcomb College, Tulane University, New Orleans, La., and Ernst C. Abbe, University of Minnesota, Minneapolis, Minn.—The pistillate inflorescence of *Leitneria floridana* Chapm. is a "spike-like" ament. The small flowers are sessile and solitary in the axils of relatively large, spirally arranged bracts, each floret being flanked by two smaller secondary bracts. Surrounding the base of the ovary of each floret is a perigon made up of small tepals. The presence of the secondary bracts, as well as the evidence furnished by a study of the vascular anatomy of the inflorescence and florets, suggests by analogy with the Betulaceae that tertiary florets were present in the phylogenetic history of the plant. The staminate inflorescence is a true ament, consisting of 40 to 50 prominent spirally arranged bracts, in the axils of which are situated the units of the inflorescence. These units have been generally interpreted as single flowers, but the study of their vascular anatomy furnishes evidence that each unit is a cymule composed of three florets.

✓ FLORAL ANATOMY OF THE GENUS *GENTIANA*. Alton A. Lindsey, American University, Washington, D. C.—*Gentiana* comprises four distinct types on the basis of the number of carpel traces leaving the stele and

the number of carpel bundles derived from these main traces. The former criterion substantiates Kusnezow's division of the genus into the subgenera *Eugentiana* and *Gentianella*, the latter marked by strong original fusion of the adjacent ventrals. Sections Coelanthae and Pneumonanthae show seven distinct traces to each carpel, while the remaining eight sections of *Eugentiana* possess three traces to each carpel. In *Gentianella*, the fused ventrals separate in the section Crossopetalum when the body of the ovary is reached, but remain fused in the other sections. The calyx traces in Crossopetalum originate on eight radii by fusion of adjacent calyx laterals. These variations in the carpel vascularization are considered to indicate the broad phylogenetic affinities within the genus.

✓ THE TYPES OF STIGMAS IN THE JUGLANDACEAE. Wayne E. Manning, Smith College, Northampton, Mass.—There are five types of stigmas in the Juglandaceae. (1) The slender, elongated, carinal type, with the stigmatic area on the inner surface of each style arm (*Juglans*, *Pterocarya*, *Platyocarya*). This type is usually described as characteristic of the family. (2) The short, subglobose, carinal type, with the stigmatic surface on the outside of each style arm or extending cap-like over the tip (*Alfaroa*, *Engelhardtia* (*Oreomunnea*) *pterocarpa*). (3) The short, 4-lobed, intermediate-commissural type (*Engelhardtia chrysolepis*). (4) The elongated, commissural type, with the stigmatic area on the edge of each style arm (*Engelhardtia spicata*, et al.). (5) The elongated commissural type, with the stigmatic papillae covering all parts of the style arms (*Carya*). In this genus there is, in addition a stigmatic disk. The subglobose stigma is probably the primitive type in the family, and there have been three lines of evolution: toward the elongated carinal type, toward the elongated commissural type of *Engelhardtia*, and toward the elongated commissural type of *Carya*.

DEVELOPMENT AND HISTOLOGY OF FRUIT OF THE SOUR CHERRY FROM PRE-BLOOM TO FRUIT RIPENING. H. B. Tukey and J. Oran Young, N. Y. State Agricultural Experiment Station, Geneva, N. Y.—During the period 18 days before full bloom, increase in the ovary wall of the sour cherry is due almost entirely to increase in number of cells. During Stage I (rapid enlargement for 20 days following full bloom) cell division predominates in the first part and cell enlargement in the latter part. Cells of the stony pericarp attain maximum size; cell divisions in the pericarp are few, if any, after this stage is completed. During Stage II (retarded development for 16 days) cell walls of the stony pericarp thicken and harden rapidly, while those of the fleshy pericarp undergo little change. During Stage III (rapid enlargement to fruit ripening) the cells of the fleshy pericarp undergo enormous enlargement, and the epidermis and hypodermal layer are stretched tangentially. The stony pericarp may be further divided into an inner and outer layer, the former being derived largely from inner epidermis. The fleshy pericarp

may be divided into inner, outer, and middle regions, in addition to the hypodermal layer and the outer epidermis. Division, enlargement, and differentiation of the cells of these tissues, and factors responsible for the shape of cells at maturity are discussed.

STRUCTURE OF THE CELL WALL OF THE AVENA COLEOPTILE DURING GROWTH. *Thomas Kerr and Kenneth V. Thimann, Bureau of Plant Industry, Washington, D. C., and Harvard University, Cambridge, Mass.*—When thin walls of growing cells are stained with congo red, fine details of cellulose structure may be observed under the polarizing microscope. This is due, apparently, to the fact that the dye precipitates as needle-like, submicroscopic crystals in the intercellular spaces; the dye crystals being oriented and birefringent. By the use of this method, we have studied the structure of the cell walls in the *Avena* coleoptile and the changes taking place during growth. The walls of growing cells are fairly complex, consisting apparently of several layers of cellulose. Thus in each side wall of the parenchyma cells there are at least two sets of crossing cellulose orientations, giving the impression of left and right handed spirally wound fibrils, with each set approximately at 70° to the axis of the cell, or to the axis of growth. The angle which these cellulose threads make with the cell axis may vary from cell to cell and even along the length of a single cell, but no significant changes in angle have been detected during growth. It is difficult to interpret these two sets of orientations in terms of present theories of wall growth, practically impossible in terms of intussusception.

FURTHER NOTES ON THE ANOMALOUS SECONDARY THICKENING IN THE MONOCOTYLEDONAE. *Vernon I. Cheadle, Rhode Island State College, Kingston, R. I.*—In the examination of additional material collected in Cuba, very early stages in the formation of adventitious roots were obtained. These early stages confirm a previous tentative conclusion by the author that such roots arise from the same growth ring which produces the typical secondary tissue on its inner side. Specific types of growth rings in such secondary tissues were previously reported as being characteristic of particular species. Examination of more material of these same species, collected from different localities, indicate that this is not true in all cases. Apparently the growth ring patterns are even more variable than heretofore supposed.

EMBRYOGENY OF JUNIPERUS COMMUNIS. *Phyllis Cook, University of Illinois, Urbana, Ill.*—Fertilization takes place in June, and one, two, or three eggs in each complex are fertilized. The complete proembryo consists of about twelve cells arranged in three more or less definite tiers. The nuclei of the upper tier are not completely walled in, and they take no part in further development; the cells of the middle tier elongate to form prosuspensors which push the embryo of the lowest tier out of the archeogonial complex into the gametophyte tissue. Each proembryo consists of four embryonic units each having a prosuspensor and an embryo initial, so that the number of

embryonic units is four times the number of original zygotes. All the units grow evenly into the gametophyte, so that at the very beginning of sporophytic life, there is keen competition among individuals. The embryo initials elongate to form uninnucleate tubes which grow with equal rapidity and then intertwine before cutting off new cells. When the new cells are cut off, they in turn elongate, forming a second set of embryonal tubes. If a few among these gain on the rest, they may form multicellular embryos; but if, as is again usual, there is still a tie among a number of units, the second tubes intertwine and cut off cells that also elongate. This process may be repeated an indefinite number of times. As each new set of embryonal tubes develops, those preceding it begin to collapse; so that the entire elongated complex resembles a much twisted and tangled rope of spaghetti. Three or four embryos may become multicellular, but it is rare to find more than one mature embryo in a seed. Since the number of sets of embryonal tubes is indefinite, the time before the embryos become multicellular varies. Two growing seasons are required for full development of the embryo.

SEEDLING ANATOMY AND CLASSIFICATION IN THE GRAMINEAE. *Paul Weatherwax, Indiana University, Bloomington, Ind.*—On the basis of the anatomy of the seedlings, grasses fall into three general classes. The correlation between this method of grouping and conventional classifications of the family has been investigated in more than 100 genera. Some tribes, such as the Chlorideae, Hordeae, Paniceae, Andropogoneae, and Tripsaceae, show great uniformity in seedling types in so far as they have been examined. Others, such as the Agrostideae, Aveneae, and Festuceae, are more diverse. In a few instances, different species of a single genus fall into different classes.

TOTAL LENGTH OF STEM DEVELOPED BY A SINGLE SEEDLING OF CUSCUTA. *H. L. Dean, State University of Iowa, Iowa City, Iowa.*—In connection with current studies the actual length of stem developed by a known single seedling of *Cuscuta Polygonorum* has been measured. The many branches of this stem totaled 2406 feet in length. Experimental methods and aspects of conduction will be briefly discussed.

DISTRIBUTION AND DEVELOPMENT OF TOBACCO ROOTS. *L. J. Gier, Campbell College, Buie's Creek, N. C.*—A number of plants were removed from the beds by the soil block washing method and from the field by the trench method. Drawings and measurements were made of the roots systems and the plants dried for shoot: root ratio studies. The ratio was found to be near 10:1 through the entire season with a consistently high correlation between the dry weights of shoots and roots. Mature plants were found to have about 1400 feet of roots. Soil type seemed to have little effect on the root pattern, but depth of plowing did change the distribution of feeders.

A NOTE ON THE DECIDUOUS SHOOTS OF TAXODIUM DISTICHUM. *G. L. Cross, University of Oklahoma, Norman, Okla.*—There are contrasting opinions concerning the interpretation of the deciduous shoots of

Taxodium. Older writers attempted to establish their homology with the dwarf shoots of *Pinus*, a view which has been discarded in at least one recent paper. The present preliminary study indicates that the problem may be complicated by the existence of more than one type of deciduous shoot in *Taxodium*. Four types of deciduous shoots, with respect to time and place of origin, have been found. One of these types arises exogenously, another endogenously, and two of them are formed "pseudocendogenously". With respect to time of origin, the four types vary from early spring to middle summer. The exogenous shoots differ structurally from the other types. The difficulty of interpreting deciduous shoots of such diversity, in terms of structures found in *Pinus*, is discussed. It is hoped that the problem may be clarified by detailed morphological and experimental studies on *T. distichum* now in progress at the University of Oklahoma. *T. ascendens* Brong., a form with acicular leaves recently found in McCurtain County, Oklahoma, and heretofore apparently unreported for the state, is receiving comparative treatment.

✓A COMPARATIVE HISTOLOGY OF SOME OF THE LAMINARIALES.—Arlo I. Smith, *McMurry College, Abilene, Tex.*—In an attempt to clear up considerable confusion as to the nature of sieve tubes in the Laminariales the author presents a comparative histology of the holdfast, stipe, transition region, and blade of *Laminaria saccharina*, *Cymathere triplicata*, and *Costaria costata* of the Laminariaceae, *Nereocystis Luetkeana* of the Lessoniaceae, and *Pterygophora californica* and *Alaria tenuifolia* of the Alariaceae.—All cells of the plants are of the same type but vary apparently because of internal environment. The epidermal meristem exists in all plants studied, and all cells of the plant are gradations from the typical isodiametric meristematic cells.—Pits are present in all cells of the plants and in greatest abundance in the transverse walls. They are nearly always arranged in a single circle composed of 5 to 25 pits, each varying in diameter 3 to 15 microns. The only exception to the circular arrangement of pits is in *Cymathere* in which the pits are scattered indiscriminately. Pit membranes are not perforated and are not sieve tubes. Of the plants investigated, only *Nereocystis* contains true sieve plates which are present in addition to pits in circular arrangement.—Microchemical tests indicate that callose is formed quite generally throughout cells of the medulla. Cell wall material is apparently a pectose-like material accompanied by varying quantities of cellulose.

NEW RESEARCH METHODS FOR THE STUDY OF ECONOMIC PLANTS. Walter T. Swingle, *Bureau of Plant Industry, Washington, D. C.*—The direct study of economic plants is beset with grave difficulties which can best be removed by a thorough study of the whole group of plants to which the cultivated genus belongs. Wild relatives of important crop plants are greatly in demand for modern plant breeding work, since by using them new disease-resistant or other-

wise superior varieties are often created. The absence in this country of nearly all the relatives of many important crop plants makes it necessary to have recourse to the great herbaria of the world. New methods are discussed for utilizing to the full this vast store of plant material. Up-to-date taxonomic monographs of all groups of plants which contain important crop plants are shown to be fundamentally important for modern plant breeding by bringing to light all wild relatives, by showing approximately the degree of relationship of such plants to their wild relatives, and by telling where to find them and what are their distinguishing taxonomic characters.

THE RELATION BETWEEN CELL DIVISION, CELL ENLARGEMENT, AND GROWTH RATE. Edmund W. Sinnott, *Columbia University, New York, N. Y.*—In a small-fruited race of *Cucurbita* studied, cell division in all tissues of the ovary except ovules and epidermis ceases at about the same time, when the ovary has reached approximately one-fourth of its final diameter. Later growth is entirely by cell expansion. Daily measurements show that growth proceeds at a constant percentage rate until the fruit has reached about half its final diameter. There is no change in rate of growth at the time when cell division ceases. During the period when cell division is occurring throughout the ovary, it proceeds at the same rate in all regions (placental region, inner wall, outer wall, and epidermis), although these regions are growing at markedly different rates because of differences in cell enlargement. The rate of growth of the ovary and its parts thus seems to be independent of the occurrence or rate of cell division within it.

THE SHAPE OF COMPRESSED LEAD SPHERES OF UNIFORM DIAMETER. James W. Marvin, *Columbia University, New York, N. Y.*—For many years surface tension has been considered an important factor in the determination of the shape of undifferentiated cells aggregated into tissues, and the orthic tetrakaidecahedron has been suggested as the figure which represents the fundamental shape of such cells. In an attempt to determine the effect of contact and pressure on uniform spheres, lead shot were subjected to varying pressures from one merely sufficient to cause a slight flattening at the points of contact to one sufficient for the elimination of all interstices. The range in the number of contacts resulting from these pressures varied from slightly more than 8 contacts for the least pressure, to 14.17 contacts for the pressure at which the elimination of the interstices occurred. The average figure was very nearly 14-sided but was not the orthic tetrakaidecahedron.

VOLUME-SHAPE RELATIONSHIPS IN LEAD SHOT AND THEIR BEARING ON CELL SHAPES. Edwin B. Matzke, *Columbia University, New York, N. Y.*—Cells in undifferentiated tissues have been shown by Lewis to have an average of approximately fourteen contacts, while Marvin has demonstrated the same for compressed lead shot of uniform size, eliminating interstices. To analyze the problem of contact and pres-

sure relationships and its bearing on cell shapes still further, selected lead shot of two diameters, one twice that of the other, were mixed in equal and then in varying proportions, by volume, and compressed to eliminate all spaces. If the proportions were equal, the small shot became roughly dodecahedral, while the large shot were compressed into polyhedra having nearly twenty-six faces. If a greater proportion of the larger shot was used, the number of faces on the small shot became still less, while that of the larger shot dropped toward fourteen. If a larger proportion of the small shot was used, the number of contacts of the small shot approached fourteen, while that of the large shot rose to about thirty. The bearing of these data on cells of varying sizes in tissues is obvious.

THE THREE DIMENSIONAL SHAPE OF THE PITH CELLS OF *EUPATORIUM PURPUREUM*. *James W. Marvin, Columbia University, New York, N. Y.*—The shape of the pith cells of *Eupatorium purpureum* was studied by means of a new technique, and three-dimensional models of the cells were constructed. This new technique made it possible to construct the models directly from the cells without the use of serial sections. One hundred of these cells were found to have an average of 13.36 contacts per cell. The cells were not regular polyhedra, but in their surface-volume relationships, while not as economical as the orthic tetrakaidecahedron and the rhombic dodecahedron, they approached these figures surprisingly closely.

PALEOBOTANICAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PALEOBOTANICAL SECTION
OF THE BOTANICAL SOCIETY OF AMERICA, RICHMOND, VIRGINIA,
DECEMBER 28 TO 30, 1938

THE FRUCTIFICATIONS OF ARCHAEOPTERIS. C. A. Arnold, *University of Michigan, Ann Arbor, Mich.*

GALLATIN FOSSIL FOREST. P. A. Young, *Texas Agricultural Experiment Station, Jacksonville, Tex.*—A little known forest of petrified *Sequoia* trunks was explored for nine summers beginning in 1926 in the Gallatin Mountains near the northwest corner of Yellowstone National Park. Here many of the *Sequoia* trees protrude vertically from the agglomerate rock. They occur on mountain ridges at altitudes of 8000 to 10,000 feet. Probably these *Sequoias* grew in the Laramie or Eocene periods. Presumably volcanic rains of rock buried at least eight successive layers of *Sequoia* forests, now visible on one mountain. Exposure of the wood to water solution silicified it, perfectly preserving the wood structure. The yellow trunks are 1 to 12 feet in diameter and 1 to 30 feet tall. One fragment looks rotted, and another was apparently tunneled by insects before petrification. A pine cone of rock shows seeds. Leaf prints resembling willow, magnolia, pine, and fern abound in pumice near the petrified trees. In the neighboring Madison valley, fragments of ulmaceous (Oligocene?) wood are beautifully silicified, showing details of structure.

CHARACTERISTIC PLANT MICROFOSSILS OF DES MOINES COALS OF IOWA. L. R. Wilson and E. A. Coe, *Coe College, Cedar Rapids, Iowa.*—Macerated coals from numerous exposures in the Des Moines Series of Iowa have been studied microscopically. Many spores are present in the coals, but only the smaller forms have been critically studied. Some of these are widely distributed in the series. Descriptions of the species and their stratigraphy are discussed.

MEDULLOSA DISTELICA, A NEW SPECIES OF THE ANGLICA GROUP OF MEDULLOSA. James M. Schopf, *Illinois State Geological Survey, Urbana, Ill.*—*Medullosa distelica* sp. nov. and petioles and roots associated with it are described. The stem possesses two highly asymmetric steles, a band-like internal periderm and fleshy decurrent leaf bases comparable to those of *M. anglica*. A few points—viz., the asymmetrical growth of steles, the leaf traces, and location of stelar protoxylem—are compared with these characters in other species. The type of asymmetric growth of secondary wood may be significant within this group for taxonomic purposes. Although *M. distelica* is less complex in its gross stelar anatomy than *M. anglica*,

this condition is regarded as derived rather than as a primitive feature.

The *Medullosa* "form-cycles" are discussed briefly. The older species which include the *M. anglica* "form-cycle" and the new American species are considered sufficiently distinct from the rest of the genus to constitute a subgeneric group designated as *Anglicana*. Another American specimen illustrated earlier by Reinhardt Thiessen shows sufficient agreement with *M. anglica* to be described as a variety of that species and indicates best how closely American and European forms are related.

THE EMBRYO OF CORDATES. W. C. Darrah, *Harvard University, Cambridge, Mass.*—Recently pyritic coal balls have been found in the Des Moines Series of Iowa. More than 800 such nodules have been cut and peeled. Among the fifty species thus far discovered, there is a small, samaropsoid seed which is very abundant. Several of these seeds contain well preserved dicotyledonous embryos. This is the first Paleozoic embryo thus far recognized.

THE FEMALE GAMETOPHYTE OF A CARBONIFEROUS SELAGINELLA. W. C. Darrah, *Harvard University, Cambridge, Mass.*—*Selaginella Amesiana* is a remarkably well preserved fossil plant from Mazon Creek, Illinois. The strobilus is composed of megasporangia bearing four spores each. Many of these megasporangia have the gametophytes endosporially developed, and these structures show cell walls, cell-plates, nuclei, nucleoli, and occasional mitotic figures.

A LARGE PSEUDOSPOROCHNUS FROM THE DEVONIAN OF NEW YORK. R. B. Schultes, *Harvard University, Cambridge, Mass.* (Introduced by W. C. Darrah.)

A PRELIMINARY REPORT ON THE FLORA OF LOUISIANA LIGNITE. Clair A. Brown, *Louisiana State University, University, La.*—Several outcrops of lignite belonging to different geological formations occur in Louisiana. The lignite under investigation came from the strata of the Vicksburg group (Oligocene) and the outcrop is located on the banks of Sugar Creek near Rosefield, Catahoula Parish, Louisiana. This lignite macerates easily and is rich in spores and pollen grains. The most characteristic and easily recognized spores are similar to those of species of *Lygodium* and *Ophioglossum*. Pollen grains similar to those of *Sequoia* are frequent. Many of the pollen grains have yet to be identified.

PHYSIOLOGICAL SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION
OF THE BOTANICAL SOCIETY OF AMERICA, RICHMOND, VIRGINIA,
DECEMBER 28 TO 30, 1938

EFFECTS OF HEAT ON YEAST. I. RATE OF DEATH OF CELLS WHICH SURVIVE MODERATE TREATMENT. *Thomas F. Anderson and B. M. Duggar, University of Wisconsin, Madison, Wis.*—When a suspension of yeast is exposed to a temperature of 50°C. for increasing lengths of time, an increasing number of cells lose the ability to divide and to form visible colonies on nutrient agar. Death rate curves have been obtained. In the present experiments it is established that a fraction of those cells which survive exposure to heat die within 10 hours of incubation at 32°, in the absence of nutrient. This fraction increases from zero for untreated controls to as much as 95 per cent for yeast exposed for one hour. The lag in appearance of colonies plated out on agar immediately after exposure to heat also increases with increasing exposure, but this lag disappears after 10 hours of incubation. The significance of these results is discussed.

GROWTH AND CELL STRUCTURE IN THE FIRST INTERNODE AND COLEOPTILE OF *AVENA* AS AFFECTED BY RED, GREEN, BLUE, AND VIOLET RADIATION. *G. S. Avery, Jr., P. R. Burkholder, and H. B. Creighton, Connecticut College, New London, Conn., and University of Missouri, Columbia, Mo.*—*Avena* seedlings were grown in water in complete darkness and in light of various wave lengths and intensities. The spectrum regions used were: red, as obtained with a mazda lamp and Schott Rg2 filter, and green, blue, and violet, as given with type H₂ mercury arcs and suitable Schott glass filters. The maximum intensity for each color of light was 44 ergs/cm²/sec. Measurements of the final length of the first internode and coleoptile were made after the first foliar leaf had burst through the coleoptile (8 days at 80°C.). Results: In darkness, the first internode grew to a greater length than the coleoptile, whereas, under light of all intensities and qualities studied, the coleoptile grew to a greater length than the first internode. Growth of the first internode was reduced appreciably by low intensities of all wave lengths: at equated intensity, red was most effective in inhibiting elongation, green and blue were next with violet following. High intensity light inhibited growth of the first internode more than the coleoptile. Cell size and cell number were correlated with length of the first internode.

AUXIN CONTENT OF EMBRYOS AND ENDOSPERMS OF GERMINATING MAIZE. *G. S. Avery, Jr., H. B. Creighton, and C. W. Hock, Connecticut College, New London, Conn.*—Maize grains were soaked in water eight hours, then placed on moist filter paper and put in a dark room at 25°C. A fresh set was started each day. At the end of six days twenty typical grains were selected from each group. Embryos were separated from endosperms, and auxin was extracted by the following methods: (1) the ether method of Boy-

sen Jensen, (2) the alcohol method of Avery and Strain, (3) the diffusion technique of Went. In (1) the auxin content of both embryos and endosperms increased during the first day of germination; on succeeding days it fell off rapidly in the embryos, whereas the endosperms had their maximum content on the second day with a more gradual decrease from there on. In (2) the dormant embryos and endosperms had approximately as great a hormone content as those after one day of germination. Otherwise results were comparable with those in (1). In (3) the endosperms showed a high auxin content on the second day of germination followed by a very gradual decrease; in the embryos, however, no auxin could be detected after the first day.

A CONSTANT TEMPERATURE CONSTANT HUMIDITY CHAMBER TO OBTAIN THE NECESSITY OF EXPENSIVE CONTROL ROOMS FOR PHYTOHORMONE TESTS. *G. S. Avery, Jr., H. B. Creighton, and C. W. Hock, Connecticut College, New London, Conn.*—Such a chamber will readily care for 6 to 8 doz. test plants per day and may be placed in an ordinary photographic darkroom. The operator works with only hands and forearms in the chamber, hence is undisturbed by the relative humidity of 56-88.

STORAGE OF ELM SEEDS. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.*—Elm seeds in open containers at room temperature or 5°C. gradually declined in germination power up to ten months of storage and after fifteen months were not viable. Germination of seeds sealed in an atmosphere of air at room temperature was fairly good after four months of storage but practically nil after seven months. If the storage vial contained oxygen, very little germination was obtained after four months, while partial vacuum permitted germination after fifteen months. Sealed storage at 50°C. in air, oxygen, and partial vacuum, as well as open or sealed storage at a temperature below freezing, were favorable for retention of vitality up to fifteen months. Seedling production in soil was found to be increased by pre-treating in moist granulated peat moss at 5°C. for one month or by soaking in water in light for twenty-four hours prior to planting.

STORAGE OF SOME FLOWER SEEDS. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York.*—The germination of air-dry seeds of the regal lily was much reduced by two years of storage at room temperature in open or sealed containers, but when the moisture content was reduced, the germination power was only slightly decreased by six years of storage. A temperature below freezing kept these seeds viable for six years regardless of the other storage conditions. Aster seeds

stored at room temperature or at 5°C. in open containers remained viable for one and one-half years, but if sealed in air or in a partial vacuum, they gave good germination after two and one-half years. If these seeds were stored below freezing, open or sealed containers were equally effective in maintaining vitality up to two and one-half years. Reducing the moisture content prolonged the life of the seeds at room temperature. Dandelion and verbena seeds have shown the same general response to storage conditions as those of aster. Air-dry seeds of pansy, sweet pea, and *Venidium* were stored successfully for two years when sealed at 5°C.

A FURTHER REPORT ON THE STORAGE OF VEGETABLE SEEDS. Lela V. Barton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—Seeds of carrot, eggplant, lettuce, onion, pepper, and tomato have been stored for six years. Reduction of moisture content of the seeds to approximately seven per cent of their dry weight was sufficient to keep the seeds (except pepper) viable when stored at room temperature. No reduction in moisture content was necessary for successful storage for six years in a room with a temperature below freezing. Plants grown from six-year-old seeds kept under favorable storage conditions appeared normal in every respect. Preliminary experiments indicated that seeds can be removed from storage and packeted for market and kept for at least two months without impairing their germination power. Lettuce seeds can be induced to germinate at high temperatures which are ordinarily prohibitive by pre-treatment on moist filter paper at 20°C. for sixteen hours.

EXPERIMENTS ON THE METABOLISM OF VITAMIN B₁. James Bonner and E. R. Buchman, California Institute of Technology, Pasadena, Calif.

PRODUCTION OF GROWTH-PROMOTING SUBSTANCE BY BACTERIA GROWN IN CULTURES CONTAINING SINGLE AMINO ACIDS. Paul R. Burkholder, University of Missouri, Columbia, Mo.—For the purpose of studying production by bacteria of growth substances active in the *Avena* test, as commonly used for detection of phytohormones, pure cultures of *Aerobacter aerogenes* and *Escherichia coli* were grown in glycerol agar containing single amino acids (1 part in 1,000 of medium) as the only source of nitrogen. Inoculated and sterile tubes of media were kept at 33.0°C. for two or four weeks, and then tests were made for growth substance in the media according to a standard *Avena* curvature technique. Seventeen of the amino acids were found to permit activation of the culture media, supporting bacterial growth, the sterile controls showing no growth-promoting property. Tryptophane alone became somewhat active upon autoclaving, but was further activated in the bacteria cultures. Growth inhibition was found with 1/1000 tryptophane cultures, but upon further dilution at the time of testing, this medium also exhibited strong growth promotion.

GROWTH PROMOTING SUBSTANCE FROM TRYPTOPHANE AND VITAMIN C IN VITRO. Paul R. Burkholder, University of Missouri, Columbia, Mo.—Studies on the

activation of tryptophane by vitamin C were made by mixing these two substances in aqueous solution, incubating for different lengths of time, and then testing with *Avena* coleoptiles. Concentrations of the order of 1 part of substance in 4,000 parts of distilled water were studied. Under standard conditions either substance alone gave little or no indication of growth activity in the phytohormone test, while mixtures yielded marked growth-promoting properties. Since tryptophane and "evitamic acid" exist in plants, and tryptophane can be converted readily to the active indole acetic acid, the implication is that some such mechanism may be concerned in the formation of growth substances in plants.

THE RESPIRATION OF BAKER'S YEAST IN VARIOUS CONCENTRATIONS OF DEXTROSE AND CYANIDE. Barry Commoner, Harvard University, Cambridge, Mass.—The respiration rate (R) of baker's yeast in various mixtures of dextrose and KCN was determined in Warburg respirometers. The relation of rate to dextrose concentration was a hyperbola, approaching a maximum R at .008 M dextrose. In 10^{-6} M to 2×10^{-4} M KCN, R was not inhibited at low dextrose concentration, but its maximum was lower than without KCN, and occurred at less than .008 M dextrose. Thus, KCN inhibited R only in the higher dextrose concentrations. At a given concentration of KCN, the percentage inhibition depended on the concentration of dextrose, and thus on the value of R in absence of KCN. The maximum rate decreased proportionally with log [KCN]. At 10^{-7} M, KCN had a stimulating effect, raising the value of R always to the maximum, independent of dextrose concentration.

THE USE OF NICOTINIC ACID IN THE CULTURE OF EXCISED PLANT PARTS. Ray F. Dawson, Columbia University, New York, N. Y.—Excised tobacco shoots were cultured in light and in darkness in 0.0007 M solutions of nicotinic acid and in water, both at pH 4.0. With nicotinic acid in the light and in the dark, leaf dry weight did not decrease appreciably, water content increased, growth continued at the stem tips, and the total solution absorption was increased 100 per cent. All plants in the light absorbed from 40 to 70 per cent more culture solution than did those in the dark. Plants in water both in light and in darkness lost in leaf dry weight, contained less water, and produced little new growth. Nicotinic acid hydrochloride was more effective than the free acid in increasing water content, dry weight, and the absorption of culture solution. However, the high acidity of the dissociated acid salt apparently caused injury to the stem tissue. The sodium salt was relatively ineffective. Total nitrogen determinations were made to show whether or not the nicotinic acid had been conducted to the leaves.

FACTORS CAUSING EARLY DEVELOPMENT AND VERNALIZATION. H. G. du Buy, University of Maryland, College Park, Md.—Chemical treatments which cause early flowering indirectly and directly were studied. Solutions of growth substances were compared with dusts of various carriers. The range of tolerance is

wider in the last, because the powders give off the regulators less rapidly than the solutions. Mixtures with substances which improve penetration (urea, thiourea, etc.) increase the uptake and translocation as measured on oat coleoptiles with the marker method. Seed treatments change root and shoot growth and, consequently, the time of flowering. Increased root initiation resulting from increased uptake of mixtures was found by the use of thiourea-naphthalene acetate mixtures. Thiourea solutions increased root elongation of seedlings of tomato, lettuce, etc. Root tissue cultures showed that thiourea compares favorably with vitamin B₁ in its root elongation capacity. Mixtures and thiourea decrease damping-off by action upon seedlings and fungus. Some effect of thiourea was noticed on shoot initiation. Phenyl-ethyl-barbiturate and phenyl-2-sec.-amyl barbiturate caused pronounced shoot development in roses. Increased shoot elongation is caused by thiourea-naphthalene acetate mixtures, as shown by the oat test, and by phenyl-ethyl barbiturate, phenyl-2-sec.-amyl barbiturate, and phenyl-ethyl malonic acid, a decomposition product. Subsequent cultivation of treated plants showed that the changes in development can cause early flowering, dependent on their cultivation in nutrient solutions deficient in Ca, K, and N. The results obtained with extracts of flowers are still variable.

RESPONSE OF ADIANTUM CUNEATUM TO THE PHOTO-PERIOD. John K. Edwards, *University of Pennsylvania, Philadelphia, Pa.*—Spores sown in November produced normal heart-shaped prothallia by February 1. At this time daily light exposures of 4, 6, and 8 hours, normal day, and normal day plus mazda light until 11:00 P.M. were started. Sex organs appeared on numerous prothallia from each light treatment during the week of April 18. During the week of May 9 young sporophytes appeared on gametophytes from all light treatments except the 4 hour; in the latter case they appeared on May 28. On September 25, the sporophytes receiving normal day and normal day plus illumination were from 4-6 inches high, well branched, and very leafy; sori were beginning to appear also, and by October 15 the sporangia and spores were mature. On October 28 the sporophytes receiving the shorter periods of illumination were still very small and in no case showed developing sori. Restricted vegetative growth of the sporophytes subjected to short photoperiods offers a possible explanation of the failure of spore formation under these conditions.

FAT METABOLISM IN THE SOYBEAN SEED. H. C. Eyster, *University of South Dakota, Vermillion, S. D.*—Soybean seeds germinated at room temperature show an increase of ether extract in percentage of dry weight during the first two days. After the second day and sometimes earlier there is a gradual decrease in the ether extract followed by a more rapid decrease during the later stages of germination. It is not definitely known whether the increase in ether extract during the first two days signifies an increase in fat

content. There is no apparent change in the iodine number of the soybean oil during seed germination and during the early growth of the seedling. A slight increase in the saponification number of the oil of the germinating seeds occurs. There is no change in the quantity or quality of oil as seed becomes older. The oil from 1925 and 1926 Manchua variety soybean seeds occurred in practically the same percentage of dry weight and had practically the same iodine number and saponification number as those harvested in 1932 and 1933. It is not known why fatty seeds are viable for a shorter time than starchy seeds.

RESPONSE OF LETTUCE SEEDLINGS TO 7600 Å RADIATION. Lewis H. Flint and Charles F. Moreland, *Louisiana State University, Baton Rouge, La.*—In studying the effects of spectral radiation on the germination of light-sensitive lettuce seed several years ago, it was found that a band of radiation at 7600 Å had a definite and pronounced inhibitory influence on germination (Flint and McAlister, *Smithsonian Misc. Coll.*, Vol. 94, No. 5, 1935). Further studies of the effect of this radiation are here described.

THE EFFECT OF CERTAIN FUNGICIDES AND ENVIRONMENT ON THE RATE OF TRANSPIRATION OF TOMATO PLANTS. Arthur C. Foster, *United States Horticultural Station, Beltsville, Maryland.*—The effect of copper phosphate, zinc sulfate, and copper sulfate in 4-4-50 spray mixtures on the rate of transpiration of large, mature tomato plants was determined concurrently with the effect of widely varying environmental conditions—namely, soil moisture, soil nutrition, air temperature, and light duration. The combined test and spray periods were from 67 to 83 days' duration. The effect of these factors on rate of transpiration was as follows: (1) zinc sulfate and copper sulfate had no effect; (2) copper phosphate caused a slight increase of 6 per cent; (3) superphosphate applied to the soil as 0-12-0 at 1 ton per acre caused a slight increase; (4) added increments of nitrogen to the soil caused a very marked decrease; (5) potash had little effect; (6) increasing amounts of soil moisture caused corresponding increases; (7) increasing air temperature also caused a corresponding increase; (8) plants grown under long day periods transpired more per gram of dry weight of plant than those grown under short days.

HEARTWOOD: ITS FORMATION AND CHARACTERISTICS. Eloise Gerry, *U. S. Forest Products Laboratory, Madison, Wis.*—In a preliminary survey of the subject the principal criteria for distinguishing heartwood from sapwood (color, odor, taste, pH, extractive content, occurrence of tyloses, susceptibility to sap stain by fungi, insect attack, permeability, shrinkage, strength, heat conductivity, absence of living cells, appearance in ultra-violet light, staining characteristics, etc.) are enumerated and evaluated. The characteristics of "ripenwood," "wet wood," "internal sapwood," and other zonate formations are discussed. Silvicultural control, by partially inhibiting or stimulating heartwood formation will be possible with a clear understanding of the correlations between heart-

wood formation and tree age, size, and spacing, and the effects of climate and soil.

SUGAR EXCRETION THROUGH PETIOLAR HAIRS IN IMPATIENS SULTANI. *Miriam G. Groner, Bucknell University, Lewisburg, Pa.*—Droplets of sugar solution are excreted through the petiolar hairs of *Impatiens Sultani*, under all conditions favorable to photosynthesis. Whenever external conditions are such that these droplets are not immediately washed away, or eaten by insects, they gradually increase in size, and lose water through evaporation until granules of crystalline sugar are formed. These granules are clean, white in appearance, and taste like cane sugar. Specific rotation, molecular weight, and qualitative tests with Foulger reagents indicate that these crystals are composed mostly of sucrose, mixed possibly with small portions of dextrose. At the same time that sugar is being excreted, starch is being formed in large quantities within the leaf. The quantity of sugar excreted varies with the amount of sunlight available. The ability of *I. Sultani* to rid itself of excess sugar is offered as an explanation for the unusual adaptability of the species to varying environmental conditions.

THE CAUSE OF NATURAL PARthenOCARPY. *F. G. Gustafson, University of Michigan, Ann Arbor, Mich.*—Data are presented on auxin concentration in the ovaries from flower buds from varieties of fruits that naturally develop parthenocarpically and from those varieties of the same species that have to be pollinated for fruit development to take place. Conclusions are drawn from these data as to the cause of natural parthenocarp.

AUXIN DISTRIBUTION IN FRUITS. *F. G. Gustafson, University of Michigan, Ann Arbor, Mich.*—Auxin concentration is reported for ovules, seeds, placentae and pericarps of many kinds of fruits. The report covers work on fruits of different stages of development and fruits produced parthenocarpically by chemicals. This is followed by a discussion of the data with respect to normal fruit development.

CONTROL OF BUD GROWTH AND INITIATION OF ROOTS AT THE CUT SURFACE OF POTATO TUBERS BY TREATMENT WITH GROWTH-REGULATING SUBSTANCES. *John D. Guthrie, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Treatment of non-dormant potato tubers with neutralized indoleacetic acid or alpha naphthaleneacetic acid inhibited the growth of buds and induced roots to grow on the cut surface. Roots were initiated on the surface of pieces cut in such a way as to have no bud tissue or skin. When treatment with potassium alpha naphthaleneacetate was followed by treatment with the dormancy breaking chemical, ethylene chlorohydrin, the inhibited buds sprouted promptly. With solid CO₂ and ether, substances active on *Avena* coleoptiles could be extracted in greater amount from the tissue after breaking the natural dormancy with ethylene chlorohydrin than from untreated controls. This may be due to an unequal destruction of auxin during extraction. Treatment of cut pieces of naturally dormant tubers with potassium indoleacetate did not break their

dormancy, although concentrations were tried in the range from 0.2 to 3200 mg. per liter and for periods of 4 to 72 hours.

THE PHOTOSYNTHESIS OF ENTIRE APPLE TREES AS INFLUENCED BY THE AGE AND EXPOSURE OF THE FOLIAGE. *Arthur J. Heinicke, Cornell University, Ithaca, N. Y.*

IDENTIFICATION OF APPLIED GROWTH SUBSTANCES. *A. E. Hitchcock and P. W. Zimmerman, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—Indole acetic and indole butyric acids recovered from above and below treated regions up to 24 days after treatment were identified by the Winkler and Peterson method and were physiologically active. Indole butyric and naphthaleneacetic acids were recovered and identified by X-ray analysis. This constitutes direct evidence that applied growth substances move longitudinally in either direction from regions treated with lanolin or aqueous preparations, thus disproving the polarity concept as applied to these acids. These substances are much more stable in plant tissue than previously claimed. There is no necessity for postulating that they act indirectly by activating or regulating the movement of hypothetical substances. They act more as chemical stimulants than specific hormones. The principal differences in their action are quantitative and not qualitative. Extraction of the ethyl ester of indole butyric acid (m.p. 40.5°C.) as described for treated tissue, yielded a residue (m.p. 38°C.) free from a hydrolyzed acid form. The possibility that esters may be recovered from treated plants in the same form applied, and not as acids, is being tested. These direct methods of identification offer unlimited possibilities for testing the validity of numerous theoretical explanations for the action of growth substances.

NEW OR MODIFIED CHLOROPHYLLS OF DATURA STRAMONIUM. *O. L. Inman and A. F. Blakeslee, C. F. Kettering Foundation, Antioch College, Yellow Springs, Ohio and Carnegie Inst., Cold Spring Harbor, N. Y.*—Chlorophylls *a* and *b* have been examined in thousands of green plants and it has been generally concluded that these were the only functional chlorophylls in the living plant. These new or modified chlorophylls (*Datura* Pale-7) are the result of X-ray treatment of the seed about six years ago. Since that time the plants have reproduced themselves but needed considerable more care in their earlier stages of growth. Examination of the chlorophylls show: Spectroscopic differences in water triturates, extracted chlorophylls and pheophytins, but normal spectra for chlorin *e*, rhodin *g*, and phylloerythrin. The percent nitrogen in the molecules was about normal, magnesium was present in the molecule. The usual chemical tests for these chlorophylls were normal. The chlorophylls are clearly functional in the photosynthetic mechanism. The present tentative conclusion is that Pale-7 chlorophylls are isomeric forms of normal chlorophyll *a* and *b* and are from recessive mutants.

EFFECTS OF 3-INDOLE ACETIC ACID ON THE OUT-GROWTH OF ROOTS FROM ISOLATED PORTIONS OF BRYO-

PHYLLUM LEAVES. Samuel Kaiser and Harry G. Albaum, Brooklyn College, Brooklyn, N. Y.—Circular portions of standard area, each containing a single notch, were cut out from various regions of *Bryophyllum* leaves. These were placed in moist chambers and continually subjected to contact with solutions of 3-indole acetic acid. In the case of each concentration (0.0067 mg./l., 0.67 mg./l., and 67.0 mg./l.), comparable portions from sister leaves were treated with tap water. A fourth set of leaves, both treated with tap water, served as an index of normal variation. Determinations of number and total length of roots growing out from these isolated portions were made over an eighteen-day period. The results follow: (1) The lowest concentration showed no appreciable effect on root number. The intermediate concentration produced a temporary and small, but significant, increase in root number, and the highest concentration effected a marked, lasting increase in root number. (2) Total root length was apparently unaffected by treatment with the lowest concentration; the intermediate concentration produced a marked, temporary stimulation during early growth; and the highest concentration effected a significant inhibition, which appeared not to endure. (3) In both treated and untreated portions, a marked gradient with regard to root number and root length was apparent, the apical leaf portions showing more active growth than those more basally situated in the original leaf.—A continued study, now in progress, on the relative amounts of growth hormones native to various portions of the leaves of *Bryophyllum* may help to explain some of the observations here presented.

THE USE OF THE DROPPING MERCURY KATHODE AS A RAPID METHOD FOR THE DETERMINATION OF SOIL OXYGEN. Kenneth S. Karsten, University of Wisconsin, Madison, Wis.—The electrolytic cell formed by a dropping mercury kathode and an anode made up of mercury is ideal, since the current-voltage curves obtained with it are absolutely reproducible. The apparatus can be constructed economically from materials readily available in most laboratories. The current-voltage curve for a 0.1 N KCl solution containing 0.05 per cent gelatin, 0.05 per cent thymol, and dissolved oxygen is characterized by a slow rise followed by a sharp rise, which is in turn followed by a second slow rise. The difference in current between the two parallel portions of the curve is proportional to the oxygen concentration. After the characteristics of the curve have been determined, the instrument is standardized against the Winkler method. The KCl-gelatin-thymol solution is boiled to remove oxygen and cooled in glass-stoppered bottles under water. A soil sample is added to one of each pair of these bottles and the resulting mixture is agitated until all the soil gas is dissolved. The concentrations of oxygen in this suspension and in the original boiled solution are determined by the dropping mercury kathode. By suitable calculations the amount of oxygen contained in the sample of soil can be ascertained.

THE ENLARGEMENT OF THE COTTON BOLL. Thomas Kerr, Bureau of Plant Industry, Raleigh, N. C.—There are two phases in the development of the cotton boll, a period of enlargement and a period of maturation. Enlargement takes place for 15–24 days after flowering, the period varying with variety and environment. The single-celled cotton fibers elongate as long as the fruit and seed are growing. Differences in fiber length in different varieties may be correlated with varying rates of elongation and different times at which growth ceases. At the end of the enlargement period, secondary wall deposition is initiated at approximately the same time in (1) the innermost layer of the carpel wall, (2) the fibers, (3) the palisade layer of the seed coat. The initiation of wall thickening proceeds in a wave. Thus in the palisade layer of the seed coat, wall thickening is first seen in cells around both the micropylar and chalazal ends of the seed, gradually spreading throughout the rest of the palisade layer. Enlargement is still taking place when wall thickening is first seen. The enlargement of the fruit following fertilization occurs during the same period as the growth of the endosperm, but enlargement of the embryos continues for a much longer time.

THE PHOSPHATE DEBT OF DIATOM CULTURES. Bostwick H. Ketchum, Harvard University, Cambridge, Mass., and Woods Hole Oceanographic Institution, Woods Hole, Mass. (Introduced by K. V. Thimann).—Phosphorus deficient cells are formed when cultures of the marine diatom, *Nitzschia Closterium*, are grown in the light in a medium containing no phosphate. When transferred to a medium containing phosphate and placed in the dark, these cells absorb phosphate rapidly for about ten hours, after which time no further absorption occurs. The phosphate debt incurred is directly related to the length of time the cells have been grown in the light in a phosphate deficient medium and is, therefore, a measure of the deficiency to which the cells have been subjected. The phosphorus absorbed to satisfy the debt combines with water-soluble organic compounds in the cells.

SPECTRAL SENSITIVITY OF SPORES AND SPORIDIA OF USTILAGO ZEAE TO MONOCHROMATIC ULTRA-VIOLET LIGHT. Ernest W. Lunden, University of Missouri, Columbia, Missouri.—Spores and sporidia of *Ustilago zeae* were treated with monochromatic ultra-violet radiation obtained from a mercury lamp and a crystal quartz monochromator. After incubation, the spores and sporidia were observed microscopically to determine the fraction surviving. Survival curves for both spores and sporidia are similar, being sigmoid in shape. No killing is observed at 3130 Å by a dose of 1,500,000 ergs/mm.² The doses required to kill 50 per cent of the spores at some of the wavelengths are as follows: 3022 Å–405,000 ergs/mm.²; 2652 Å–15,000 ergs/mm.²; 2399 Å–40,000 ergs/mm.²; 2300 Å–34,700 ergs/mm.² Comparative data for sporidia are: 3022 Å–105,000 ergs/mm.²; 2652 Å–1,760 ergs/mm.²; 2399 Å–3,350 ergs/mm.²; 2300 Å–1,020 ergs/mm.²; 2260 Å–610

ergs/mm.² Using 50 per cent killing as a criterion, there is a maximum sensitivity for both spores and sporidia at about 2650Å. A minimum sensitivity occurs at about 2400Å. Below 2400Å the sensitivity of the spores increases slightly, but the sensitivity of the sporidia increases rapidly. The increase in sensitivity below 2400Å is greater for the sporidia than for any other microorganism previously reported. In comparing the sensitivity of the sporidia with the absorption of nucleic acid, it is found that the two curves are similar, the sensitivity increasing more abruptly than the absorption of the nucleic acid below 2650Å. The sporidia of *U. zeae* are from 4 to 35 times more sensitive than the spores, depending on the wavelength.

APHIDS, AUXONES, PSEUDOGALLS, AND GALLS *George K. K. Link and Virginia Eggers, University of Chicago, Chicago, Ill.*—The mealy plum aphid (*Hyaloperatus arundinis*), collected in 1937 and 1938 from leaves of *Phragmites communis*, was used to study the relation of growth substances which occur in and on aphids to pseudogall and gall formation. Sections of infected leaves show that this aphid punctures the phloem cells, supposedly rich in auxins. Using Van Overbeek's procedure, it was found that ether extracts of the aphid contain high concentrations of one or more auxins, which, when heated with HCl and NaOH, respectively, at 100°C., behave like auxentriolic acid, and much lower concentrations of one or more auxins which behave like indole-3-acetic acid. These findings indicate that the phloem of *Phragmites communis* contains auxones (including presumably auxin a) and suggest a way of obtaining plant auxones without cutting or killing the tissues. They also support the hypothesis that dysauxony (involving both auto-auxones and heteroauxones) is part of the causal complex of pseudogall and gall formation. It is not concluded that auxins are the only auxones occurring in aphids or effective in cecidogenesis.

EFFECT OF SOME VITAMINS ON GROWTH OF LUPINUS ALBUS SEEDLINGS. *David I. Macht and Mary Lou Grumbein, Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.*—Elongation of roots of *Lupinus albus* seedlings grown under standard conditions in plant-physiological solution was compared with that of another series in weak solutions of vitamins A, B, C, and D dissolved in such saline. Vitamins B and C were employed in concentrations of from 1:10,000 to 1:250,000. Vitamins A and D, being very little soluble, were first dissolved in 95 per cent ethanol, and solutions containing 0.5 and 1.0 per cent alcohol were made in plant-physiological saline. Vitamins B and C inhibited root growth, while A and D stimulated it. Extremely interesting was the effect of combinations of vitamin solutions. Some exhibited synergism—i.e., a potentiation of toxicity; others revealed antagonistic effects; and still others exerted a toxicity equivalent to a summation of their individual component effects. These findings agree with those obtained by Tislowitz (Science Progress, October, 1937) and others study-

ing vitamins on animals and speak against indiscriminate administration of artificial vitamin mixtures in clinical cases.

FORMATION OF GLUCOSIDES BY PLANT TISSUE FROM INTRODUCED AGLUCONS. *Lawrence P. Miller, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—In connection with investigations on the breaking of the rest period by ethylene chlorohydrin it has been found that both gladiolus corms and potato tubers form a glucoside hydrolyzable by emulsin from the absorbed ethylene chlorohydrin. Through preparation of the tetra-acetate, the glucoside formed has been shown to be B-(2-chloroethyl)-d-glucoside in both gladiolus and potato. Experiments with a variety of other plants and plant parts, which had absorbed ethylene chlorohydrin either as vapor or from the nutrient medium, have shown that the gladiolus and potato are not exceptional, but that the ability to form a B-glucoside from ethylene chlorohydrin is very general. Since there is reason to believe that B-(2-chloroethyl)-d-glucoside is less toxic to plants than ethylene chlorohydrin, these results support the view of those who consider that glucoside formation in plants may serve as a detoxication mechanism.

MOVEMENT OF WATER THROUGH LIQUID LAYERS AGAINST A GRADIENT. *W. J. V. Osterhout and J. W. Murray, Rockefeller Institute for Medical Research, New York, N. Y.*—In certain models designed to imitate living cells the behavior of water is opposite to expectation. The simplest setup is as follows: Guaiacol saturated with water is placed in the bottom of a U-tube; water saturated with guaiacol is placed in the arms of the U-tube so as to rest on the guaiacol. Since the whole system is in equilibrium, there is no movement of water. Upon lowering the activity of the water in one arm of the U-tube by adding trichloroacetic acid, the water might be expected to move into this arm. However, just the reverse happens. Water and acid move from the solution of acid to the opposite arm of the tube. This might be ascribed to the formation of hydrates (i.e., the acid carries water of hydration with it) or to "salting in" of water into the guaiacol. Similar results were obtained with non-electrolytes. This appears to differ from anomalous osmosis, which depends on the presence of pores and of electrolytes.

THE PATH OF FLUORESCIN MOVEMENT IN THE RED KIDNEY BEAN, PHASEOLUS VULGARIS L. *Edward M. Palmquist, Cornell University, Ithaca, N. Y.*—Schumacher reported that fluorescein moves in the phloem. Later, Rhodes concluded that it moves in the xylem, but ordinarily is not detectable there without raising the pH of that tissue, and that the apparent movement in the phloem results from lateral diffusion into that tissue from the adjacent xylem. Cross sections of untreated twigs, representing twenty-five genera, were examined with a fluorescence microscope. Most of these showed a trace of yellow fluorescence in the xylem. These sections were then exposed to ammonia vapor. The xylem of all of them fluoresced with a brilliant yellow light similar to that of fluorescein.

These twigs had not been treated with fluorescein. The xylem of young bean plants does not fluoresce, either with or without the ammonia treatment. Fluorescein was applied to a series of thoroughly watered bean plants. Twenty-four hours later, cross sections of the stems showed fluorescence in the phloem, but not in the xylem. Exposure to ammonia vapor failed to produce fluorescence in the xylem. It is concluded that fluorescein moves in the phloem of bean plants.

A TECHNIC FOR THE CYTOLOGICAL STUDY OF LIVING PLANT CELLS. H. S. Reed and E. T. Eltinge, *University of California, Berkeley, Calif.*—By a method recently devised by the authors, it is possible to grow excised root tips of plants under aseptic conditions for microscopical and microchemical study. Seeds can be taken from fruits under aseptic conditions and germinated on filter paper in test tubes. When the root has grown to a length of 1 or 2 cm., the terminal 3 to 5 mm. of the root is cut off and transferred under aseptic conditions to a hanging drop of nutrient solution or to an agar slant. The hanging drop culture permits direct observation under the microscope. The other requires less frequent transfers but permits one to get straight roots if the tubes are kept upright. In either case there are many exfoliated cells from the growing region of the root which can be transferred and are available for examination of physiological processes under the microscope. Vital dyes and other reagents may be introduced under the cover glass or in any way desired for the study of other problems connected with cell physiology.

METHODS OF EXTRACTING PLANT JUICES FOR VITAMIN C DETERMINATIONS. Mary E. Reid, *National Institute of Health, Washington, D. C.*

TREE TEMPERATURES AND THERMOSTASY. E. S. Reynolds, *Washington University, Saint Louis, Mo.*—A continuous, approximately four-year, automatic record of the temperatures of a tree in the center and cambium zones and also of the concurrent air temperature are reported. When the tree first reached 0°C., a long period of adjustment occurred before the internal temperature followed the downward trend of the air temperature. This is due to the high thermal capacity of wet tissues. At high temperatures the tree temperature varied inversely with that of the air because of the great absorption of heat from the tissues due to the high latent heat of vaporization during the conversion of water into water vapor. This internal evaporation is associated with the development of a partially evacuated condition as a result of foliar transpiration. At moderate temperatures also, considerable vaporization and cooling of the tissues took place, which largely accounts for the slow adjustment between tree and air temperatures. These two thermostatic actions within the tree tend to protect the living tissues from injury under critical temperature conditions. The demonstration of extensive vaporization in the tree tissues raises anew the question of the significance of water vapor in the transfer of water through plants.

GERMINATION AND GROWTH OF SOME ROCK GARDEN PLANTS. Elora M. Schroeder and Lela V. Barton, *Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.*—The seeds studied exhibited several types of germination behavior. Seeds of *Calochortus macrocarpus*, *Camassia Leichtlinii*, and *Lewisia rediviva* need no pre-treatment but must be kept at low temperatures (1° to 5°C.) for germination. *Draba alpina* seeds germinated at a daily alternating temperature of 10° to 30°C. after pre-treatment for two months at 1°, 5°, or 10°C. *Ramondia pyrenaica* and *Primula obconica* seeds were light-favored, while those of *Genista prostrata* gave much better germination after treatment with concentrated sulphuric acid had removed the hard coat. Seeds of *Campanula barbata*, *Campanula garganica*, *Draba aizoides*, *Gentiana lagodechiana*, *Hypericum coris*, *Mimulus Langsdorffii*, *Penstemon ambiguus*, and *Primula pulverulenta* germinated well over a wide range of temperatures. Different soil mixtures and temperatures were used for further development of the seedlings. *Draba aizoides* and *Draba alpina* required a cool greenhouse (15°C.) for growth and flowering.

QUANTUM EFFICIENCY OF PHOTOSYNTHESIS IN CHLORELLA. John F. Stauffer, *University of Wisconsin, Madison, Wis.*—Determinations were made of the quantum efficiency of photosynthesis in two species of *Chlorella*. The algal cells were suspended in a nutrient solution and the gas exchange during photosynthesis determined with a Barcroft differential manometer. The light employed was chiefly a narrow band in the red (ca. 6500 Å) at intensities near the compensation point. Experiments were made at temperatures of 10, 15, 20 and 25°C. Under these conditions the efficiency of the process was less than that reported by other investigators previous to 1930.

EXPERIMENTS ON THE ROOTING OF PISUM CUTTINGS. Lewis A. Thayer, *Centenary College, Shreveport, La.*—In seeking to discover why peas (Alaska), used for rooting test at the California Institute of Technology, root poorly during the summer months, an attempt was made to correlate rooting with sensibility to auxin (curvature tests) and auxin content (indicated by growth increments). Correlation was insignificant. It was discovered that peas grown in light rooted better than controls grown in a darkroom. Decapitated peas with 1 part indole-3-acetic acid in 100 parts lanolin applied to the cut surface rooted better than untreated controls in the same flat. The latter were better than controls in a separate flat, indicating leaching of auxin from the roots of the treated peas. Peas watered with 1 part indole-3-acetic acid in 10 million parts of water also rooted better than controls.

THE VEGETATIVE PROPAGATION OF DIFFICULT PLANTS. Kenneth V. Thimann and Albert L. Delisle, *Harvard University, Cambridge, Mass.*—The effect of auxin on root formation has been studied mainly with plants which root, at least to some extent, without auxin. Experiments on plants which under normal conditions practically never root from cuttings show that these fall into two groups—(1) plants rooting readily

on treatment with auxin, though scarcely at all without (*Tsuga*, *Picea pungens*, *Keteleeria*), and (2) those not rooting appreciably with any auxin treatment (*Pinus*, *Picea excelsa*, *Frazinus*, *Quercus*). In the latter, the most important factor is the age of the tree from which the cutting is taken. Cuttings from young trees root readily on treatment with optimal auxin. Lateral shoots root more readily than terminal, and basal parts more readily than apical. The root-forming factor missing from old trees is neither vitamin B₁ nor sugar; if it is a substance, its amount decreases with age and its distribution varies widely within the plant. Vegetative propagation of such "difficult" plants is thus perfectly practical.

THE RELATIVE ACTIVITIES OF DIFFERENT AUXINS. Kenneth V. Thimann and Charles L. Schneider, Harvard University, Cambridge, Mass.—The activities of seven auxins were compared for straight growth of immersed sections of *Avena* coleoptiles and *Pisum* stems and for curvature of slit *Pisum* stems ("pea test"). In general, straight growth is promoted by lower concentrations than cause inward curvature, which agrees with the explanation of the "pea test" previously given. There is considerable variation from experiment to experiment, not only in absolute activity, but also in the relative activities of different substances compared to that of indole acetic acid as standard. The relative activities also differ with the test method and are not the same in *Avena* as in *Pisum*.

A MODIFIED AUXIN TEST OF HIGH SENSITIVITY. Kenneth V. Thimann and C. L. Schneider, Harvard University, Cambridge, Mass.—As previously shown, the inward curvature of halved stems ("pea test" of Went) is exhibited also by halved *Avena* coleoptiles. Since these objects are semi-tubular in cross-section, the growth of the part which lies alongside the slit tends to oppose the curvature. By slitting into four instead of two parts, most of this resistance is removed, resulting in an increase of sensitivity of from 10 to 100 times. Thus a concentration of auxin giving only 0.1° curvature in the standard *Avena* test can be readily measured. The results support completely the theory of these curvatures previously put forward (Amer. Jour. Bot. 25: 627, 1938).

OXYGEN REGULATES THE DORMANCY OF THE POTATO. Norwood C. Thornton, Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y.—Dormancy of freshly-harvested potatoes is easily broken by storage in 2 to 10 per cent of oxygen, and it is prolonged by storage in higher percentages of oxygen. Freshly-harvested potatoes do not normally sprout because the skin or periderm is too permeable to oxygen, but with ageing of the tuber this tissue becomes less permeable to oxygen so that sprouting will take place in progressively higher percentages of oxygen. Moist storage conditions facilitate, and dry storage conditions retard the development of the periderm. The rest period can be shortened by removing the skin, because the "wound cork" tissue that develops very rapidly is more effective than the normal periderm in

retarding the passage of oxygen into the tuber. Sprouting of cut potatoes can be temporarily retarded by placing in high percentages of oxygen until the "wound cork" tissue develops to a greater thickness. Sprouting of one-eye pieces of dormant or non-dormant potatoes can be retarded by frequently removing the "wound cork" tissue thus permitting the entrance of excess oxygen into the tuber.

THE RELATIVE HUMIDITY OF STOMATAL OPENINGS AND INTERCELLULAR SPACES OF SOME LEAVES. H. F. Thut, Eastern Illinois State Teachers College, Charleston, Ill.—Small portions of mature leaves were exposed to various atmospheres of known relative humidity. These were maintained by fastening the leaves over "humidity bottles" containing sulfuric acid solutions of different concentrations or pellets of sodium or potassium hydroxide. The remainder of the plant was exposed to the usual environment of the laboratory or greenhouse. Such experiments were performed on well-watered plants and plants showing definite wilting. *Lantana*, bean, petunia and sunflower were used. The water loss or absorption by the leaf was an inverse linear function of the relative humidity over the entire range from 100 per cent to near zero. The point at which the curve passed through the zero line of water loss from the leaf represents in terms of relative humidity the state of the water vapor in the stomatal openings and intercellular spaces. *Lantana* shows a point of 91 per cent relative humidity for a well-watered plant and a point of 75 per cent for a wilted plant. Corresponding values for bean were 85 and 65, for petunia 89 and 79, and for sunflower 94 and 83.

TEMPERATURE RELATIONS IN THE GERMINATION OF DIGITARIA. E. H. Toole and Vivian K. Toole, U. S. Department of Agriculture, Washington, D. C.—Striking differences were found in response to temperature during germination of freshly harvested seed of *Digitaria ischaemum* and *D. sanguinalis*. Both species required for germination daily alternation of temperature and a long period, varying from 9 to 30 weeks. *D. ischaemum* germinated most rapidly under alternating high temperatures of 20°–40°C., and progressively slower at the lower temperatures, 20°–35°, 20°–30°, and 15°–25°. In contrast, the rate of germination of *D. sanguinalis* decreased progressively for the temperature alternations 20°–30°, 20°–35°, and 20°–40°, but was also slowest at 15°–25°. Pre-chilling the moist seed at 3° greatly hastened germination of both species, but the period of chilling which was required for prompt germination was much shorter when followed by germination at the most favorable temperature for the species than when followed by a less favorable germination temperature.

ULTRA-VIOLET TRANSMISSION BY POLLEN OF ZEA MAYS. Fred M. Ueber, University of Missouri, Columbia, Mo.—In order to formulate a satisfactory interpretation of genetic data concerning the effects of ultra-violet radiation on corn pollen, it was first necessary to secure a quantitative estimate of the radiation actually incident on the effective material—

i.e., the chromosomes or genes. Measurements of the transmission through the wall and contents of pollen grains were made in two ways: (1) by means of a Geiger-Mueller counter with a cadmium photo-electric surface, a condensing lens being used to focus a minute spot of monochromatic radiation on the object, and (2) by means of an ultra-violet microscope, employing platinum step-filters at the photographic plate for calibration purposes. The photographic densities were determined with a microphotometer. Transmission curves indicating selective absorption by both the pollen wall and the pollen grain contents were obtained. Absorption determinations were also made on various pollen extracts.

POTENTIALLY UNLIMITED GROWTH OF EXCISED PLANT CALLUS IN AN ARTIFICIAL NUTRIENT. *Philip R. White, Rockefeller Institute for Medical Research, Princeton, N. J.*—Callus obtained from proliferating pro-cambial tissue of a hybrid *Nicotiana* (*N. langsdorffii* × *N. glauca*) has been kept growing in culture in a nutrient similar to that earlier developed for cultivation of excised roots through 40 one-week passages. Cultures regularly increased two or more fold (often ten fold) in volume each week, giving a theoretical dilution of the original material to less than one molecule per culture ($2 \text{ fold} = 2^{-40} = \text{ca. } 1 \times 10^{-12}$, $4 \text{ fold} = 4^{-40} = \text{ca. } 1 \times 10^{-24}$, $10 \text{ fold} = 10^{-40}$). The environmental complex can therefore be considered adequate for unlimited growth of this material. The growth habit was similar to that obtained in callus attached to the plant. On a semi-solid medium the tissue remained undifferentiated, as far as could be detected macroscopically, but formed rudimentary buds in a liquid medium. With the possible exception of Gautheret's cultures in which growth was very slow, this appears to be the first time that an undifferentiated callus mass has been maintained in continuous and undiminished growth in artificial culture for a long enough period and through enough passages to assure its capacity for unlimited growth. This seems to be the first true plant "tissue culture" in the sense of Carrel.

THE BOUND WATER CONTENT OF NATIVE PRAIRIE SPECIES IN RELATION TO ENVIRONMENTAL CONDITIONS. *Warren Whitman, North Dakota Agricultural Experiment Station, Fargo, N. D.*—The bound water of the leaf tissue of the principal grass species in four different grassland types in western North Dakota was determined by calorimeter technique weekly during July, August, and early September for two seasons. Soil moisture determinations to a depth of two feet were made at weekly intervals at each station. Precipitation, evaporation, and temperature and relative humidity records were obtained at one central station throughout the two seasons. As the season progressed, temperature and evaporation increased, relative humidity and soil moisture decreased, and the general trend of the species in all habitats was toward somewhat higher osmotic values, higher percentages

of the total water in the plant held as bound water, and yet lower absolute values for bound water per gram of dry matter. Exceptions were noted in some species in which a marked decrease in amount of bound water per gram of dry matter occurred, while in other cases the bound water remained relatively stable throughout the season. Some of the more obvious differences between species are related to the rooting habit and depth of available soil moisture. In other cases the behavior apparently must be attributed to physiological differences between species.

THE FACTORS INFLUENCING THE NUMBER OF STOMATA. *L. Edwin Yocum, George Washington University, Washington, D. C.*—An increase in the number of stomata with the increasing height on the plant has been described by various authors. The writer finds this to be true for seedling oaks, but not for grown trees. A study of the factors which determine the number of stomata was made. Moonflower plants were grown in soil with optimum moisture and in soil with the moisture near the wilting coefficient. In some cases the water content was changed from optimum to low or vice versa, when the plants were partially grown. In each case, the influence of optimum soil-moisture content compared with low moisture content was clearly demonstrated by the water requirement, the size of the leaves, the number of leaves, the height of the stem, the green weight, and the dry weight of the plants. The stomatal index appears to be unaffected by changes in soil moisture content. Plants grown in the greenhouse in the autumn and those grown in the spring showed that seasonal conditions altered the stomatal number. The stomatal number was always influenced more by soil moisture than by height on the plant.

CONTROLLING DAMPING-OFF OF TOMATO SEEDLINGS WITH LIGHT, AND CHEMICALS ON SEED AND SOIL. *P. A. Young, Tomato Disease Laboratory, Texas Agricultural Experiment Station, Jacksonville, Texas.*—Hot beds and cold frames here have cloth covers that decrease sunlight from 1000 foot-candles to 100–300 foot-candles as measured by a Weston exposure meter. Many farmers grow seedlings under sheets. These chlorotic seedlings are very susceptible to damping-off by *Pythium* and *Rhizoctonia*. Damping-off was extreme in seedlings receiving only 5 to 100 foot-candles of light in laboratory experiments in the last 3 years. Damping-off was decreased with 300 foot-candles of light. Higher intensities of sunlight were needed for growing normal tomato seedlings. Dusting tomato seed with Cuprocid, Metrox, Zinc Oxide, or Vasco 4 or soaking seed in solutions of Semesan or New Improved Ceresan decreased damping-off under conditions strongly favoring this disease. Carbon bisulphide was ineffective, Semesan was fair, Cuprocid "54" was good, and Formaldehyde and Chloropierin were very effective when used in treating soil to control post-emergence damping-off of tomato seedlings.

SYSTEMATIC SECTION

ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE SYSTEMATIC SECTION
OF THE BOTANICAL SOCIETY OF AMERICA, RICHMOND, VIRGINIA,
DECEMBER 28 to 30, 1938

AN INADEQUATELY DESCRIBED SPECIES OF ASCLEPIAS. Roland M. Harper, *University of Alabama, Tuscaloosa, Ala.*—The species in question has been known for nearly 150 years, but it differs from the rest of the genus in having follicles drooping instead of erect, as commonly described. Its seeds are somewhat different from those of other species, pointing to a different method of dissemination, doubtless correlated with its exceptional habitat.

ASCLEPIAS TUBEROSA VS A. DECUMBENS, AND THE SPECIES-PROBLEM. W. H. Camp, *New York Botanical Garden, New York, N. Y.*—*Asclepias tuberosa* originally was a species of the prairies and plains, extending into northern Mexico, while *A. decumbens* was probably limited to areas mostly eastward of the Appalachians. Due to the prevailing westerly winds on the expanding prairies of the post-Pleistocene xeric period, the range of *A. tuberosa* was sufficiently enlarged to come into contact with that of *A. decumbens*, at which time the two species hybridized. The comparatively recent creation of artificial prairies (fields and pastures) has given further impetus to the eastward migration and establishment of the more vigorous *A. tuberosa*, so that *A. decumbens* now exists in pure form in only a few restricted areas.

VEGETATIVE VERSUS REPRODUCTIVE CHARACTERES, AS ILLUSTRATED IN THE "GERARDIA" GROUP. Francis W. Pennell, *Academy of Natural Sciences, Philadelphia, Pa.*—Since the time of Linnaeus such emphasis has been placed upon reproductive structures as to lead to undue neglect of vegetative features. Thus, in the "*Gerardia*" group have been associated the campanulate-flowered *Aureolaria* (yellow) and *Gerardia* (*Agalinis*) (purple); the yellow rotate-flowered *Dasistoma* and *Seymeria*; and the orange tubular-flowered *Macranthera* and *Esterhazyia*. *Aureolaria*, with ample leaves of characteristic form, is eastern North American. *Gerardia*, with corollas of elaborate pattern and characteristically narrow leaves is of temperate North and South America, primitively of the latter. Similarly, *Dasistoma* and *Seymeria*, and *Macranthera* and *Esterhazyia*, differ vegetatively, *Dasistoma* and *Macranthera* closely resembling *Aureolaria*, all in eastern North America; *Esterhazyia* through *Virgularia* connecting with *Agalinis*, all in South America; and *Seymeria* with primitive Mexican members showing corollas fundamentally like *Gerardia*. Vegetative and geographic evidence shows two series in our entire group: one North American, comprising *Aureolaria*, *Dasistoma*, and *Macranthera*; and one, originally South American, comprising *Gerardia*, *Esterhazyia*, and, less closely, *Seymeria*. While vegetative structure has remained relatively constant, in each series the flower-form has changed with differing methods of pollination, most primitive being the bee flowers of

Aureolaria and *Gerardia*, and most transformed the hummingbird flowers of *Macranthera* and *Esterhazyia*.

THE CONCEPT OF THE SPECIES. H. A. Gleason, *New York Botanical Garden, New York, N. Y.*—The concept of the species has been fixed by nearly two centuries of use and is illustrated by nearly 200,000 accepted species of flowering plants. The scope of the species is reasonably uniform. In the vast majority of cases, the concept depends on external characters which in their totality make a definite impression on the mind. The term species should be reserved for groups of this nature. For other groups, characterized by internal or microscopic structure and by physiological or genetical behavior, a different term should be used.

THE RELATION OF ANATOMY AND CYTOLOGY TO THE CLASSIFICATION OF THE LEGUMINOSAE. Harold A. Senn, *Div. of Botany and Plant Path., Science Service, Dept. of Agric., Ottawa, Canada.*—An analysis of the anatomy of the secondary xylem of two hundred genera of ligneous Leguminosae along with the determination and compilation of chromosome numbers in seventy-five genera has permitted a re-examination of the tribal classification of the family. The evidence demonstrates that the arrangement of Bentham and Hooker, and later of Taubert, is in large part a natural one but that certain groups such as the Amherstieae, Sophoreae, and Hedysyreae are highly heterogeneous anatomically and cytologically. The anatomical evidence demonstrates the close relationship between the three subfamilies Mimosoideae, Caesalpinioideae, and Papilionatae. In contrast to the anatomical uniformity of the first two subfamilies, the Papilionatae show extreme anatomical diversity paralleled by great morphological variability. The anatomical and cytological data are not only of value in determining broad phyletic trends in the family but also in some instances are of value in critical generic delimitation.

COMPARISON OF THE SEQUOIAS. John T. Buchholz, *University of Illinois, Urbana, Ill.*—The two species of *Sequoia* have been compared with respect to gross morphology and internal morphological criteria. Nearly two dozen differences appear which will be presented and discussed. Some of these features are very important in separating genera (and even families) among other conifers. The question is raised: Do these differences warrant a generic distinction between the two species?

SOME CHEMICAL PROPERTIES OF EUCALYPTI IN RELATION TO THEIR EVOLUTIONARY STATUS. James B. McNair, *818 South Ardmore Ave., Los Angeles, Calif.*—There has been orderly evolution in volatile oil characteristics of the Eucalypti which may be correlated with changes in morphological characteristics. In the

case of most primitive and "natural group" the "Bloodwoods," there is homogeneity between systematic characters and chemical properties. However, oil groups represent cross sections of genealogical tree and not necessarily genetic sequences or "natural groups" of the systematist. Views of Baker and Smith and Maiden are thus reconciled. Botanically distinct species are distinguished by their chemical constituents, and in some cases, where morphological examination shows little or no difference, chemical analysis of oil reveals existence of completely distinct varieties. Development of chemical characteristics and morphological characters do not necessarily proceed hand in hand; one may proceed more or less rapidly than the other. Oil specific gravity tends to increase with increase in plant evolution, and refractive index tends to decrease with increase in plant evolution, except where oxygenated bodies decrease in amount with increase in evolution. The number and variety of oxidation products increase, and optical rotation becomes more hevo-rotatory with advance in evolution.

A PRELIMINARY STATEMENT ON THE PHYLOGENETIC SIGNIFICANCE OF THE ANOMALOUS SECONDARY THICKENING IN THE MONOCOTYLEDONEAE. *Vernon I. Cheadle, Rhode Island State College, Kingston, R. I.*—Secondary thickening by means of a growth ring has been noted in several families of the Monocotyledoneae, chiefly in the Liliaceae as defined by Engler and Prantl. This peculiar type of enlargement may offer another factor or tool in solving the problem of the relationships among the groups of plants within the Monocotyledoneae. It has been noted by the author and others that the amount of tissue laid down by means of this thickening ring varies in the plants examined. This fact, together with the general habit of growth and with certain features of the primary body (e.g., size, presence or absence of vessels, perhaps types of vascular bundles) of the various plants involved, leads the observer on preliminary inspection to the tentative opinion that the plants with the greatest amount of such secondary thickening may be the most ancient and that these possibly are the starting points for several broad phylogenetic lines, somewhat similar in nature to those set up by Hutchinson in his treatment of the Monocotyledoneae.

COMPARATIVE ANATOMY OF THE SECONDARY XYLEM IN THE VIOLACEAE AND FLACOURTIACEAE. *Fred H. Taylor, Harvard University, Cambridge, Mass.*—A comparative study of the secondary xylem in the Violaceae and Flacourtiaceae bears out the contention of the systematist that these families, at least, form a homogeneous unit in an order marked by a lack of uniformity. Anatomically they exhibit numerous primitive features whose discovery is definitely interesting in dicotyledons placed high in the Archichlamydeae. Vessel elements are relatively long with inclined end walls, for the most-part. Multiple perforations of the scalariform type are not uncommon. Parenchyma is rare to absent and, when present, is usually scanty vasicentric. Nearly all the fibrous

elements in both families show septations, and, since their pits, though small, are definitely bordered, they must be classed as septate fiber-tracheids. Rays, in the majority of cases, are longitudinally high and of primitive types. Vessel-ray pitting is transitional scalariform to alternate; intervacular pitting mostly alternate. Gelatinous fibers are common, especially in the Flacourtiaceae. A cursory examination of the wood of the Turneraceae gives an indication that this family also may belong to the complex.

THE SIGNIFICANCE OF ANATOMY IN TAXONOMIC STUDIES IN THE JUGLANDACEAE. *Charles Heimsch, Jr., Harvard University, Cambridge, Mass.*—Recent taxonomic studies of juglandaceous inflorescences by Manning have shown that a terminal androgynous panicle, involving both male and female catkins, is the primitive type of fruiting structure for the family. The genera *Engelhardtia*, *Alfaroa*, and *Platycarya* display this type of inflorescence. In the main, anatomical studies confirm these results. *Engelhardtia* and *Alfaroa* show primitive tendencies in that they retain scalariform vessel perforations in the mature wood. In addition, vessel distribution in these genera is diffuse, the primitive arrangement. Other anatomical characters agree with these more obvious traits to reveal an organization of a higher type in *Carya*, *Juglans*, and *Pterocarya*. This accords with the more specialized inflorescence ascribed by Manning to these genera.

THE ANATOMY AND DEVELOPMENT OF THE SPIDER LILY SEED. *Muriel Whitehead and Clair A. Brown, Louisiana State University, University, La.*—The seed of the Spider Lily, *Hymenocallis occidentalis* (LeConte) Kunth, is unique. The modified integuments develop into a succulent tissue possessing chlorophyll and stomates. Four vascular bundles have been traced through the funiculus into the fleshy integuments of the ovule where each branches several times. The embryo is "incomplete" at the time of shedding, but in a period of three weeks the embryonic sporophyte develops into an elongate structure consisting mainly of cotyledon with only a small hypocotyledonary region. Germination then takes place immediately.

LAST SURVIVORS IN THE FLORA OF TIDEWATER VIRGINIA. *M. L. Fernald, Gray Herbarium, Harvard University, Cambridge, Mass.*

GEOGRAPHIC RELATIONS IN THE GENUS PHLOX. *Edgar T. Wherry, University of Pennsylvania, Philadelphia, Pa.*—While at least twice as many species of *Phlox* occur in the Cordilleran region as in the Alleghenian region, consideration of the details of distribution indicates that the genus radiated from a center northwest of the present Great Lakes. It may therefore be termed a Kewatin genus.

GRASSES NEW TO NORTH CAROLINA. *H. L. Blomquist, Duke University, Durham, N. C.*—In an intensive survey of the grass flora of North Carolina, thirty-two grasses have been found which have not been previously reported from the state. Nine of these are chance introductions which occur as weeds,

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while the majority are permanent numbers of the native vegetation. Of the latter some were to be expected within the state because of their ranges, while others represent either north or south range extensions.

OBSERVATIONS ON THE FLORA OF MOUNTAIN LAKE, VIRGINIA. *John M. Fogg, Jr., University of Pennsylvania, Philadelphia, Pa.*—An account of the vascular plants and floristic relationships of the vicinity of the Mountain Lake Biological Station, at Mountain Lake, Giles County, Virginia. The character of the terrain, with its high ridges, bald summits, bluffs, rivers, flood plains and bogs, is described and the more important geological formations indicated. The predominant character of the vegetation, which is that of deciduous Alleghanian woodland, is outlined and some of the more conspicuous plants mentioned. The various components of the flora are then analyzed, an attempt being made to distinguish between the intraneous and extraneous elements. Among the latter category, attention is directed chiefly to the more significant constituents, such as the Canadian or boreal element, the southern Appalachian element, and the Coastal Plain element. The percentage of each group, as plotted against the total number of species present, is given. Certain species which are of greater interest ecologically than phytogeographically are discussed, and mention is made of several striking omissions in the present list of plants known to the area.

THE SHALE BARREN FLORA OF WEST VIRGINIA. *Earl L. Core, West Virginia University, Morgantown, W. Va.*—A narrow strip of shale of Ordovician and Devonian age outcropping along both sides of the Virginia-West Virginia border and extending northwards across western Maryland into southern Pennsylvania supports a peculiar flora, including a considerable number of remarkable endemics which constitute one of the most interesting components of the plant life of West Virginia. The present paper summarizes the literature on the subject, so far as West Virginia is concerned, locates the Barren areas, and lists the typical species, with distribution records.

FIELD STUDIES OF VIRGINIA FLORA. *A. B. Massey, Virginia Polytechnic Inst., Blacksburg, Va.*

MIDSUMMER PLANTS OF TENNESSEE. *H. K. Svenson, Brooklyn Botanic Garden, Brooklyn, N. Y.*

FLORISTIC CHANGES WITHIN THE CITIES OF THE PRAIRIE REGION DUE TO THE GREAT DROUGHT. *Raymond J. Pool, University of Nebraska, Lincoln, Neb.*

—The profound reactions of flowering plants to the severe drought conditions of 1934 to 1938 are revealed nowhere in more vivid and startling degree and variety than within the cities of the prairie region. Drought damage has been studied as revealed by numerous indigenous and introduced species of trees and herbaceous plants commonly used for ornamental purposes. Interesting reactions are recorded in connection with the behavior of lawns also.

THE CARICES OF WESTERN NORTH AMERICA. *J. W. Stacey, California Academy of Sciences, San Francisco, Calif.*—Short history. Problems of nomencla-

ture, with special reference to phyllopody, and form and structure of the perigynia and achenes. Phylogenetic relationships within the genus. The problem of distribution. Importance of the use of the genus in ecological study, in view of the fact that it ranges in altitude from sea-level to beyond tree-limit on the mountains. The use of chemistry and chromosome counting in taxonomic study. The value of the study of the genus from a practical standpoint, such as: reclamation of marsh lands, sand-binding on the seashore or in the so-called "dust-bowls," and the presence or absence of its members as a factor in the advance or retreat of the tree-limit on mountains. Collecting, mounting, and filing in herbaria. The description of new species.

THE VASCULAR FLORA OF NEWFOUNDLAND LABRADOR.

David Potter, Clark University, Worcester, Mass.—

The area considered lies between 51°15' and 60°30' north latitude and extends through approximately 10 degrees of longitude (56° to 67°). Habitats are present ranging from sea level to mountains approximately 5000 feet in height. Our knowledge of the vascular flora is due to the activities and collections made by the early Moravian missionaries and from the collections made during several relatively recent expeditions. There are at least 550 species representing approximately 55 families. Taxonomic and phytogeographical problems are of particular interest.

SOME STOCK-POISONING PLANTS IN MARYLAND.

George B. Reynard, United States Department of Agriculture, Bureau of Plant Industry, Beltsville, Md.

—A survey of the 23 counties, including regions from the Atlantic seaboard to the Alleghany mountains, revealed, among other facts, the following:

Species of *Prunus*, *Kalmia*, *Cicuta*, *Solanum*, and *Xanthium* are of some importance throughout the state. On the "Eastern Shore," *Ornithogalum* bulbs, found in abundance, have caused death of sheep by chemical or other means. *Veratrum*, *Pteridium*, *Rhododendron*, and *Ranunculus* are of special interest in the mountainous sections.

Poisonous plants have been responsible for losses of cattle, sheep, horses, hogs, goats, and poultry. From actual cases reported, plants in at least 30 genera and 19 families have been involved. The total number, with both deadly and less toxic plants included, is at least 60 genera in 36 families.

Incidental or plants of minor importance are included in the genera *Datura*, *Robinia*, *Sorghum*, *Gymnocladus*, *Lycium*, *Melilotus*, *Ricinus*, *Aesculus*, and *Phaseolus*. *Eupatorium urticaefolium* may have caused poisoning in earlier times but is not known to be important in that respect today.

DISTRIBUTION OF SOUTHERN SALIX SPECIES. *Carleton R. Ball, Extension Service, U. S. D. A., Washington, D. C.*—*Salix petiolaris*, a species of northern glaciated areas, has been found recently in northern Georgia and southwestern Virginia. How far south in the Appalachians are *S. longipes* Wardii, *S. cordata*, and *S. discolor* found? What are the eastern limits of *S. interior* (longifolia) in the lower Mississippi

Valley? What are the altitudinal ranges of *S. nigra*, *S. sericea*, *S. humilis*, and *S. tristis*?

FURTHER NOTES ON COASTAL FLORAL ELEMENTS IN AUGUSTA COUNTY BOGS. Lloyd G. Carr, University of Virginia, Charlottesville, Va.—A large number of coastal plain species occur in Augusta County, Virginia, in bogs and grassy meadows on the western slope of the Blue Ridge at an elevation of 1600 feet. Among these may be mentioned: *Panicum longifolium*, *P. hemotinum*, *P. virgatum*, *Calamagrostis cinnoides*, *Rhynchospora gracilentia*, *Scleria reticularis* var. *pubescens*, *Carex Mitchelliana*, *Juncus scirpoides*, *Utricularia subulata*, *Xyris flexuosa*, *X. caroliniana*, *Hibiscus Moscheutos*, *Hypericum petiolatum*, *Desmodium scssilifolium*, *Eupatorium rotundifolium* var. *ovatum*, *E. verbenifolium*, *Solidago graminea* var. *polyccephala*, *S. tenuifolia*. In neighboring ponds occur *Eleocharis melanocarpa*, *Utricularia fibrosa*, *U. radiata*, *U. gibba*, *U. clandestina*, *Ericaulon septangulare*, *Oronchium aquaticum*, *Scirpus subterminalis*. An explanation of the occurrence of such distinct coastal types is correlated with the physiographic history of the provinces. These are considered relic colonies of a coastal flora once widespread in the southeastern states when the Appalachian uplands were base leveled in Cretaceous times.

FRANCIS WOLLE'S FILAMENTOUS MYXOPHYCEAE. Francis Drouet, Field Museum of Natural History, Chicago, Ill.—I had the opportunity recently to examine most of the herbarium material of filamentous Myxophyceae seen by Rev. Francis Wille (1817-1893) and undertook to match the reports of species in Wille's publications with these specimens. Wille's importance as a student and collector is such that today, more than 50 years after he began his work, our knowledge of the algal flora of the middle and southern Atlantic states still rests chiefly upon the collection which he accumulated. His interpretations of species were influenced by the theories of polymorphism of the algae made popular in the 1880's by Hansgirg, Kirchner, and others. Wille did not, as do we, have the advantage of the excellent works of Bornet & Flahault and Gomont, which constitute the points of departure for our present system of nomenclature and classification in the group. Consequently, we find it often difficult to interpret his nomenclature into a type understandable to us unless we examine the actual specimens of which he spoke. In part, our general lack of appreciation of his work is due to the bibliographic methods used in the compiling of our recent manuals, wherein we are confused with a hodge-podge of obsolete and modern nomenclature. The several thousand specimens examined are well preserved; they easily explain Wille's many novelties and his unfamiliar specific names.

NOTE ON A LARGE OSCILLATORIA IN LOUISIANA. Eben M. West, Louisiana State University, University, La.—During a course in algae given by Dr. G. W. Prescott, of Albion College, Michigan, at the Louisiana State University Summer School, field collections were made which included an *Oscillatoria* having a diam-

eter of about 80 microns. The outstanding features of this material will be indicated with lantern slides.

A STUDY OF SOME DIATOMS OF THE FAMILIES FRAGILIARIACEAE AND EUNOTIACEAE FROM BRAZIL. Ruth Patrick, Academy of Natural Sciences, Philadelphia, Pa.—The collections on which this study is based were made by Dr. Francis Drouet and Dr. Stillman Wright while connected with the Comissão Technica de Piscicultura do Nordeste. Most of the collections, which are from Northeast Brazil, are from Ceara, Para, Paraíba, Pernambuco, Maranhao, and Rio Grande do Norte. There are more species of the genus *Eunotia* than of any other genus. For the most part they are species typical of warmer waters. However, some exceptions do occur. Some interesting facts concerning the distribution of the species are noted.

VEGETATIONAL ZONES OF BRITISH GUIANA. A. C. Smith, New York Botanical Garden, New York, N. Y.—During the American Museum Terry-Holden Expedition of 1937-38 and subsequently, the speaker made botanical collections in the basins of the Essequibo and Rupununi Rivers, working in most of the natural habitats of the Colony. Three main regions are distinguishable: the alluvial strip, the rain forest, and the interior savanna and sandstone region. The rain forest, which covers approximately 90 per cent of the country, is not floristically homogeneous but consists of four or five distinct communities. The savanna, likewise, shows various types of vegetation depending upon soil and other factors.

FLORAS OF THE WORLD. S. F. Blake, Bureau of Plant Industry, Washington, D. C.—For several years Miss Alice Atwood, of the U. S. Department of Agriculture library, and the speaker have been preparing an annotated catalog of the floras of the world. The first part, containing the North American, South American, African, Australian, and Insular Floras is now nearly ready for publication. The catalog aims to furnish an annotated list of all the now useful floras and floristic works, including those in periodical literature, which list or describe the complete vascular flora (or the phanerogams only) of any region or locality, no matter how small, and to include as well all publications dealing on the same scale with economic and medicinal plants, vernacular names, and botanical bibliography. Popular works, monographs, publications dealing with only a part of the flora or of the economic plants, and work of historical value only are not included. The first part of the work includes about 2000 titles.

A BRIEF DESCRIPTION OF THE CUBAN FLORA. J. B. Carabia.—In this paper I have tried to give an idea of the different zones of vegetation in Cuba. Beginning with the xerophytic vegetation of the south coast of Oriente, I am covering the vegetation of the slopes of the mountains up to 2000 meters. I am speaking of the community of palms and grasses of the serpentine savanna of Oriente, Camaguey, Santa Clara, Havana, and Pinar del Rio, the vegetation of the "Mogotes," and the pine land of the last province,

giving also a brief idea of the semi-xerophytic coast, sand beaches and swamp vegetation of this island.

EARLIEST PUBLISHED WORK ON POLYNESIAN BOTANY. *F. R. Fosberg, University of Pennsylvania, Philadelphia, Pa.*—Parkinson's *Journal of the Voyage of H. M. S. Endeavor*, London, 1773, contains a section on plants of Otaheite (Tahiti) which has been rather generally overlooked. In it are mentioned 82 species, 71 of them by Latin binomials, the others by aboriginal names only. Five of these names can be considered validly published for the first time in this paper. Of these, three are at present rather widely used, but ascribed to other authors. The other two, if accepted, will displace two widely accepted generic names. *Sitodum* and *Aniotum* antedate, respectively, *Artocarpus* and *Inocarpus*, by three years. Both these latter are widely used names of economic plants. They are both, here, strongly recommended to the international committee on nomenclature for conservation.

SOME BOTANICAL FEATURES OF A DESERT REGION NEAR OLANCHITO, HONDURAS. *T. G. Yuncker, DePauw University, Greencastle, Ind.*—The Aguan river rises

in the Department of Yoro and flows to the Caribbean Sea through a valley which gradually widens as it nears the coast. The river is bordered by a river-bottom area (vega land) of varying width of fertile soil which is annually inundated. At some distance from the river the land rises abruptly from the vega land to a height of about sixty feet to form a plateau-like plain which gradually rises in altitude as it extends toward the foothills. In the vicinity of Olanchito and extending southwestward for several miles this tableland is semi-arid and forms a desert-like region unusual for this part of Central America. A considerable part of this area is covered with an open forest-like growth of stunted trees and shrubs with scattering large species such as the Guanacaste, Ceiba, etc. Epiphytic bromeliads, cacti, and orchids are unusually abundant. Several species of exceptionally large tree-like cacti, thirty or more feet in height, are a dominant feature of the region.

FEATURES OF THE MANGROVE VEGETATION OF SOUTHERN FLORIDA. *John H. Davis, Southwestern University, Memphis, Tenn.*